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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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TITLE OF THE INVENTION (500 characters max)

GENES INVOLVED IN PLANT FIBRE DEVELOPMENT

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☐ Application Data Sheet. See 37 CFR 1.76

☐ Loose set of figures (5 pages), diskette containing

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Respectfully submitted,

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Date 3/31/04

REGISTRATION NO. 28,678
(if appropriate)
Docket Number: 72246-PRO

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PROVISIONAL APPLICATION COVER SHEET
Additional Page

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Docket Number 72246-PRO

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Number 2 of 2

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Yingro Wu, et al

Provisional Application No.: Not Yet Known

Filed : Herewith

For : Genes Involved in Plant Fibre Development

1185 Avenue of the Americas
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March 31, 2004

Commissioner for Patents
P.O. Box 1450
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GENES INVOLVED IN PLANT FIBRE DEVELOPMENT

FIELD OF THE INVENTION

The present invention relates to polypeptides, and polynucleotides encoding therefor, involved in the regulation of fibre initiation and/or elongation in fibre producing plants. In particular, the present invention provides methods of altering fibre initiation in cotton and products thereof. The invention also relates to the use of these polypeptides and polynucleotides as markers of fibre production in plants including cotton.

BACKGROUND OF THE INVENTION

Cotton (*Gossypium hirsutum*, and to a lesser extent *Gossypium barbadense* together with other *Gossypium* species) provides about 55% of the fibre used in textile manufacturing globally and is an important contributor to world economies. The cotton fibre is probably the most elongated cell in the plant kingdom. The molecular mechanisms that control the differentiation of this elongated plant cell are still largely unknown. Although commonly called fibres, these cells are not part of the vascular tissue and arise, instead, from the ovule epidermis. Fibres of cotton are extremely long single elongated epidermal cells that develop on the outer surface of cotton ovules, reaching upwards of 5 centimetres in some species. Fibre initiation starts between a day before and up to a day after anthesis and the fibre initials begin to elongate immediately after fertilisation, ballooning out from the surface of the seed coat epidermis. After a period of elongation, secondary cell wall thickening fills the fibre with cellulose and the fibre dies and collapses to form the mature fibre that is harvested from the seeds.

In contrast to the discovery of numerous genes responsible for fibre elongation and secondary cell wall synthesis, few genes have been identified that are associated with fibre initiation. Early cytological studies showed structural changes in fibre initials occur up to three days before anthesis: including enlarged nucleoli and nuclei, as well as an increased number of Golgi complexes (Berlin, 1986). It has been proposed that the *Arabidopsis* leaf trichomes, which require at least twenty genes for normal development (Hülskamp et al., 1994), could serve as a model for elucidating the genetic mechanisms controlling cotton fibre initiation and differentiation.

One of the first genes to be characterised in controlling leaf trichomes, GLABROUS1 (GL1), encodes a member of the Myb family of transcription factors (Oppenheimer et al., 1991). An exhaustive search of a cotton ovule cDNA library recovered six novel Myb-domain genes, but none of them encoded a GL1 homolog (Loguercio et al., 1999). *Arabidopsis* trichome initiation is proposed to be controlled by a trichome promoting complex comprised of GL1, TRANSPARENT TESTA

GLABRA1 (TTG1, a WD40 protein), and GLABRA3 (GL3, a basic Helix-Loop-Helix protein). GLABRA2 (GL2, a Homeodomain protein) regulates trichome morphology and spacing and TRIPTYCHON (TRY, a Myb-like protein) mediates lateral inhibition of trichome development in cells adjacent to each trichome (Rerie et al., 1994; Walker et al., 1999; Szymanski et al., 2000; Schellmann et al., 2002; Ohashi et al., 2002). However, genes with similar functions in cotton have yet to be identified, and hence it remains speculative whether these two single celled epidermal hair systems share any common features.

There is a need for the identification and characterization of genes involved in fibre initiation in fibre producing plants such as cotton.. This will enable markers to be used to screen plants for desirable fibre traits, as well as allow for the production of transgenic plants with altered fibre production.

SUMMARY OF THE INVENTION

To identify genes that may be specific to fibre initiation, the present inventors have used mRNA from early stage fertilised ovules of wild type and 5 lintless mutants of cotton (that produce little if any fibres) to probe a cotton ovule cDNA microarray containing 10,000 cDNAs expressed around the time of fibre cell differentiation. Since pollination may already have occurred and zygote development initiated at this stage, the inventors used a separate microarray comparison between the mRNAs of the outer integument and those of the inner ovule tissues of the wild type cotton to filter out those genes that are not expressed specifically in the seed coat outer integument where the fibres are initiated. Using this strategy genes have been identified that are differentially expressed in the lintless mutants, and hence play a role in fibre initiation.

As a result, in one aspect the present invention provides a method of altering fibre initiation and/or elongation in a fibre producing plant comprising manipulating said plant such that the production of a polypeptide is modified when compared to a wild-type plant, wherein the polypeptide comprises a sequence selected from the group consisting of:

i) an amino acid sequence provided as any one of SEQ ID NO's:1 to 16; or

ii) an amino acid sequence which is at least 50% identical to any one of SEQ ID NO's:1 to 16.

Preferably, the polypeptide comprises an amino acid sequence which is at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 76%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least

97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to any one of SEQ ID NO's: 1 to 16.

In one embodiment, the polypeptide comprises a sequence selected from the group consisting of:

i) an amino acid sequence provided as SEQ ID NO: 1; or

ii) an amino acid sequence which is at least 80% identical to SEQ ID NO:1.

In another embodiment, the polypeptide comprises a sequence selected from the group consisting of:

i) an amino acid sequence provided as SEQ ID NO:2; or

ii) an amino acid sequence which is at least 80% identical to SEQ ID NO:2.

In a further embodiment, the polypeptide comprises a sequence selected from the group consisting of:

i) an amino acid sequence provided as SEQ ID NO:3; or

ii) an amino acid sequence which is at least 80% identical to SEQ ID NO:3.

In another embodiment, the method comprises recombinantly expressing the polypeptide in said plant.

In an alternate embodiment, the method comprises reducing the level of the polypeptide endogenously produced by the plant. This can be achieved by any means known in the art. One example is by exposing the plant to an antisense polynucleotide or a catalytic polynucleotide which hybridizes to an mRNA molecule encoding the polypeptide. Another example is by exposing the plant to a dsRNA molecule that specifically down-regulates mRNA levels in a cell of an mRNA molecule encoding the polypeptide.

In a further embodiment, the plant is a horticultural plant.

In a particularly preferred embodiment, the plant is a species of the Genus *Gossypium*.

In a second aspect, the present invention provides a method of assessing the potential of a fibre producing plant to produce fibre, the method comprising analysing the plant for a genetic variation in a polynucleotide associated with fibre initiation and/or elongation, wherein the polynucleotide comprises a sequence selected from the group consisting of:

i) a nucleotide sequence provided as any one of SEQ ID NO's:17 to 45; or

ii) a nucleotide sequence which is at least 50% identical to any one of SEQ ID NO's:17 to 45.

Preferably, the polynucleotide comprises a nucleotide sequence which is at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 76%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to any one of SEQ ID NO's: 17 to 45.

As the skilled addressee would be aware, the genetic variation associated with fibre initiation and/or elongation may be in the coding portion of a polynucleotide of the the invention, or may be genetically linked to be useful as a marker for fibre initiation and/or elongation.

In one embodiment, the method comprises performing an amplification reaction on nucleic acids obtained from said plant, or nucleic acids synthesized using nucleic acids from said plant as a template, wherein the production of an amplicon in said amplification reaction indicates an association with fibre producing potential.

In another embodiment, the method comprises performing an amplification reaction on nucleic acids obtained from said plant, or nucleic acids synthesized using nucleic acids from said plant as a template, wherein the lack of production of an amplicon in said amplification reaction indicates an association with fibre producing potential.

In a further embodiment, the method comprises performing a hybridization reaction on nucleic acids obtained from said plant, or nucleic acids synthesized using nucleic acids from said plant as a template, wherein a detectable signal produced by the hybridization reaction indicates reduced fibre producing potential.

In yet another embodiment, the method comprises performing a hybridization reaction on nucleic acids obtained from said plant, or nucleic acids synthesized using nucleic acids from said plant as a template, wherein the lack of a detectable signal by the hybridization reaction indicates reduced fibre producing potential.

In an alternate embodiment, the polynucleotide is mRNA and the method comprises determining the levels of mRNA of the polynucleotide in the plant ovule at, or around, anthesis.

In a third aspect, the present invention provides a method of assessing the potential of a fibre producing plant to produce fibre, the method comprising analysing

the plant for polypeptide involved in fibre initiation and/or elongation, wherein the polypeptide comprises a sequence selected from the group consisting of:

i) an amino acid sequence provided as any one of SEQ ID NO's:1 to 16; or

ii) an amino acid sequence which is at least 50% identical to any one of SEQ ID NO's:1 to 16.

Preferably, the method comprises determining the levels of the polypeptide in the plant ovule at, or around, anthesis.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:1,

ii) a polypeptide comprising an amino acid sequence which is at least 87% identical to SEQ ID NO:1, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

Preferably, the polypeptide comprises an amino acid sequence which is at least 95% identical to SEQ ID NO:1.

In a further aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:2, and

ii) a biologically active fragment of i),

wherein the polypeptide regulates fibre initiation and/or elongation.

In yet another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:3,

ii) a polypeptide comprising an amino acid sequence which is at least 54% identical to SEQ ID NO:3, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:4,

ii) a polypeptide comprising an amino acid sequence which is at least 55% identical to SEQ ID NO:4, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:5,

ii) a polypeptide comprising an amino acid sequence which is at least 50% identical to SEQ ID NO:5, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:6,

ii) a polypeptide comprising an amino acid sequence which is at least 50% identical to SEQ ID NO:6, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:7,

ii) a polypeptide comprising an amino acid sequence which is at least 79% identical to SEQ ID NO:7, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:8,

ii) a polypeptide comprising an amino acid sequence which is at least 66% identical to SEQ ID NO:8, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:9,

ii) a polypeptide comprising an amino acid sequence which is at least 95% identical to SEQ ID NO:9, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides s substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:10,

ii) a polypeptide comprising an amino acid sequence which is at least 67% identical to SEQ ID NO:10, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:11,

ii) a polypeptide comprising an amino acid sequence which is at least 55% identical to SEQ ID NO:11, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:12,

ii) a polypeptide comprising an amino acid sequence which is at least 59% identical to SEQ ID NO:12, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:13,

ii) a polypeptide comprising an amino acid sequence which is at least 77% identical to SEQ ID NO:13, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:14,

ii) a polypeptide comprising an amino acid sequence which is at least 50% identical to SEQ ID NO:14, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:15,

ii) a polypeptide comprising an amino acid sequence which is at least 64% identical to SEQ ID NO:15, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

In another aspect, the present invention provides a substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:16,

ii) a polypeptide comprising an amino acid sequence which is at least 50% identical to SEQ ID NO:16, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

With regard to the polypeptide aspects, it will be appreciated that % identity figures higher than those provided above will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the polypeptide comprises an amino acid sequence which is at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 76%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to the relevant nominated SEQ ID NO.

Preferably, the polypeptide can be purified from a species of the Genus *Gossypium*.

Preferably, the polypeptide is a fusion protein further comprising at least one other polypeptide sequence.

In a preferred embodiment, the at least one other polypeptide is selected from the group consisting of: a polypeptide that enhances the stability of a polypeptide of the present invention, a polypeptide that assists in the purification of the fusion protein, and a polypeptide which assists in the polypeptide of the invention being secreted from a cell (particularly a plant cell).

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:17;

ii) a sequence of nucleotides as provided in SEQ ID NO:18;

iii) a sequence encoding a polypeptide of the invention;

iv) a sequence of nucleotides which is at least 87% identical to SEQ ID NO:17 or SEQ ID NO:18; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:46.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:19,

ii) a sequence of nucleotides as provided in SEQ ID NO:20,

iii) a sequence encoding a polypeptide of the invention, and

iv) a sequence complementary to any one of i) to iii).

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:21;

ii) a sequence of nucleotides as provided in SEQ ID NO:22;

iii) a sequence encoding a polypeptide of the invention;

iv) a sequence of nucleotides which is at least 54% identical to SEQ ID NO:21 or SEQ ID NO:22; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:47.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:23;

ii) a sequence encoding a polypeptide of the invention;

iii) a sequence of nucleotides which is at least 55% identical to SEQ ID NO:23; and

iv) a sequence which hybridizes to any one of i) to iii) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:48.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:24;

ii) a sequence of nucleotides as provided in SEQ ID NO:25;

iii) a sequence encoding a polypeptide of the invention;

iv) a sequence of nucleotides which is at least 50% identical to SEQ ID NO:24 or SEQ ID NO:25; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:49.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:26;

ii) a sequence of nucleotides as provided in SEQ ID NO:27;

iii) a sequence encoding a polypeptide of the invention;

iv) a sequence of nucleotides which is at least 50% identical to SEQ ID NO:26 or SEQ ID NO:27; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:50.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:28,

ii) a sequence of nucleotides as provided in SEQ ID NO:29,

iii) a sequence encoding a polypeptide of the invention,

iv) a sequence complementary to any one of i) to iii).

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:30;

ii) a sequence of nucleotides as provided in SEQ ID NO:31;

iii) a sequence encoding a polypeptide of the invention;

iv) a sequence of nucleotides which is at least 65% identical to SEQ ID NO:30 or SEQ ID NO:31; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:51.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:32,

ii) a sequence of nucleotides as provided in SEQ ID NO:33,

iii) a sequence encoding a polypeptide of the invention,

iv) a sequence complementary to any one of i) to iii).

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:34;

ii) a sequence of nucleotides as provided in SEQ ID NO:35;

iii) a sequence encoding a polypeptide of the invention;

iv) a sequence of nucleotides which is at least 70% identical to SEQ ID NO:34 or SEQ ID NO:35; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:52.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:36;

ii) a sequence of nucleotides as provided in SEQ ID NO:37;

iii) a sequence encoding a polypeptide of the invention;

iv) a sequence of nucleotides which is at least 55% identical to SEQ ID NO:36 or SEQ ID NO:37; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:53.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:38;

ii) a sequence encoding a polypeptide of the invention,

iii) a sequence of nucleotides which is at least 65% identical to SEQ ID NO:38; and

iv) a sequence which hybridizes to any one of i) to iii) under high stringency conditions.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:39;

ii) a sequence of nucleotides as provided in SEQ ID NO:40;

iii) a sequence encoding a polypeptide of the invention;

iv) a sequence of nucleotides which is at least 95% identical to SEQ ID NO:39 or SEQ ID NO:40; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:54.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:41;

ii) a sequence of nucleotides as provided in SEQ ID NO:42;

iii) a sequence encoding a polypeptide of the invention;

iv) a sequence of nucleotides which is at least 50% identical to SEQ ID NO:41 or SEQ ID NO:42; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:55.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:43;

ii) a sequence of nucleotides as provided in SEQ ID NO:44;

iii) a sequence encoding a polypeptide of the invention;

iv) a sequence of nucleotides which is at least 65% identical to SEQ ID NO:43 or SEQ ID NO:44; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:56.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:45;

ii) a sequence encoding a polypeptide of the invention;

iii) a sequence of nucleotides which is at least 50% identical to SEQ ID NO:45; and

iv) a sequence which hybridizes to any one of i) to iii) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:57.

With regard to the polynucleotide aspects, it will be appreciated that % identity figures higher than those provided above will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the polynucleotide comprises a nucleotide sequence which is at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 76%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to the relevant nominated SEQ ID NO.

In a further aspect, the present invention provides a catalytic polynucleotide capable of cleaving a polynucleotide according to the invention.

Preferably, the catalytic polynucleotide is a ribozyme.

In yet another aspect, the present invention provides an oligonucleotide which comprises at least 19 contiguous nucleotides of a polynucleotide according to the invention.

In another aspect, the present invention provides a double stranded RNA (dsRNA) molecule comprising an oligonucleotide according to the invention, wherein the portion of the molecule that is double stranded is at least 19 basepairs in length and comprises said oligonucleotide.

Preferably, the dsRNA is expressed from a single promoter, wherein the strands of the double stranded portion are linked by a single stranded portion.

In a further aspect, the present invention provides a vector comprising or encoding the polynucleotide according to the invention.

The vectors may be, for example, a plasmid, virus, transposon or phage vector provided with an origin of replication, and preferably a promotor for the expression of the polynucleotide and optionally a regulator of the promotor. The vector may contain one or more selectable markers, for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian expression vector. The vector may be used *in vitro*, for example for the production of RNA or used to transfect or transform a host cell. Preferably, the vector is capable of replication in a plant cell.

Preferably, the polynucleotide is operably linked to a plant ovule or fibre specific promoter.

In another aspect, the present invention provides a vector comprising or encoding oligonucleotide of the invention or the dsRNA molecule of the invention.

In a further aspect, the present invention provides a host cell comprising a vector according to the invention.

In a further aspect, the present invention provides a transgenic plant, the plant having been transformed with polynucleotide according to the invention or an oligonucleotide of the invention.

In one embodiment, the polynucleotide is capable of expression to produce a polypeptide according to the invention.

In an alternate embodiment, the plant has been transformed such that it produces a catalytic polynucleotide of the invention, or a dsRNA molecule of the invention.

In a further embodiment, the polynucleotide, catalytic polynucleotide or dsRNA down-regulates the production of a polypeptide of the invention which is endogenously produced by the plant.

In a further aspect, the present invention provides a substantially purified antibody, or fragment thereof, that specifically binds a polypeptide of the invention.

In another aspect, the present invention provides a method of breeding a fibre producing plant, the method comprising performing a method according to the first, second or third aspects of the invention.

In a further aspect, the present invention provides a method of selecting from a breeding population a fibre producing plant with altered fibre initiation and/or elongation potential, the method comprising;

i) crossing two plants which have differing potential to produce fibre,

ii) performing a method according to the first, second or third aspects of the invention on progeny plants,

iii) selecting a progeny plant with altered fibre initiation and/or elongation potential when compared to a parent plant.

In another aspect, the present invention provides a plant produced by a method of the invention.

In a further aspect, the present invention provides a seed of a plant, transgenic or otherwise, of the invention.

In a further aspect, the present invention provides fibre of a plant, transgenic or otherwise, of the invention.

In another aspect, the present invention provides a method of identifying an agent which alters fibre initiation and/or elongation of a fibre producing plant, the method comprising

a) exposing a polypeptide which is at least 50% identical to any one of SEQ ID NO's:1 to 16 to a candidate agent, and

b) assessing the ability of the candidate agent to modulate the activity of the polypeptide.

In a further aspect, the present invention provides a method of identifying an agent which alters fibre initiation and/or elongation of a fibre producing plant, the method comprising

a) exposing a polypeptide which is at least 50% identical to any one of SEQ ID NO's:1 to 16 to a binding partner which binds the polypeptide, and a candidate agent, and

b) assessing the ability of the candidate agent to compete with the binding partner for binding to the polypeptide.

Preferably, the binding partner is detectably labeled.

In a further aspect, the present invention provides a method of identifying an agent which alters fibre initiation and/or elongation of a fibre producing plant, the method comprising

a) exposing a polynucleotide encoding a polypeptide which is at least 50% identical to any one of SEQ ID NO's:1 to 16 to a candidate agent under conditions which allow expression of the polynucleotide, and

b) assessing the ability of the candidate agent to modulate levels of polypeptide produced by the polynucleotide.

In another aspect, the present invention provides a method of identifying an agent which alters fibre initiation and/or elongation of a fibre producing plant, the method comprising

a) exposing a polynucleotide which is at least 50% identical to any one of SEQ ID NO's:17 to 45 to a candidate agent, and

b) assessing the ability of the candidate agent to hybridize and/or cleave the polynucleotide.

As will be apparent, preferred features and characteristics of one aspect of the invention are applicable to many other aspects of the invention.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The invention is hereinafter described by way of the following non-limiting Examples and with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

Figure 1. Expression profiles of the candidate genes measured by microarrays. The plots on the left side (column A) represent the results from the DP16 time course experiment and the values plotted are the ratios relative to 0 dpa. The plots on the right side (column B) represent the results of multi-time point Lintless 4A/DP 16 comparisons and the values plotted are the ratios of Lintless 4A/DP16 at the corresponding dpa. The genes showing similar expression profiles are plotted together.

Figure 2. RT PCR of GhMyb25 with β-tubulin as control.

a. Different tissues from DP16. –Co: negative control, reaction without reverse transcriptase; O: 0 dpa ovule; P: petal; L: leaf; R: root; S: stem.
b. Ovule and fibre from DP16. The numbers indicate the corresponding dpa.
c. Ovule from 3 mutants. The numbers indicated the corresponding dpa.
d. Southern blotting of the c. hybridised with an ON035F4 probe.

Figure 3. RT-PCR of GhHD1 with β-tubulin as control. –Co: negative control, reaction without reverse transcriptase; –4 to 4: DP16 ovules of various stages (dpa) as indicated by the corresponding number; 8O: 8 dpa ovule of DP16 after fibres being removed; 8F: 8 dpa detached fibres of DP16; 0-5B: 0 dpa ovule form mutant 5B; 0-4A: 0 dpa ovule from mutant 4A; L: leaf; P: petal; R: root; H: hypocotyl.

Figure 4. Histograms of relative DNA contents of ovule epidermal cells and fibre cells.

KEY TO THE SEQUENCE LISTING

SEQ ID NO:1 - Partial homeodomain like protein encoded by GhHD1 cDNA (clone ON033M7).

SEQ ID NO:2 - Myb transcription factor like protein encoded by GhMyb25 cDNA (clone ON035F4).

SEQ ID NO:3 - Partial cyclin D3 like protein encoded by GhCycD3;1 cDNA (clone OCF07F4).

SEQ ID NO:4 - Partial protein encoded by GhFaE1 cDNA (clone ON035N9).
SEQ ID NO:5 - Possible partial protein encoded by GhFU1 cDNA (clone ON035C9).
SEQ ID NO:6 - Possible partial protein encoded by GhFU2 cDNA (clone ON005F1).
SEQ ID NO:7 - α-expansin like protein cDNA (encoded in part by clone Pfs14x).
SEQ ID NO:8 - Partial protein encoded by GhTMTP cDNA (clone CHX015K18).
SEQ ID NO:9 - Sucrose synthase gene encoded by Ghsus cDNA (clone CHX002C10).
SEQ ID NO:10 - Partial protein encoded by GhLTP cDNA (clone ON033M19).
SEQ ID NO:11 - Protein encoded by GhLTP2 cDNA (clone OCF010D8).
SEQ ID NO:12 - Partial protein encoded by GhMyb25-like cDNA (clone ON038N8).
SEQ ID NO:13 - Protein encoded by GhRD22 cDNA (clone OCF005C10).
SEQ ID NO:14 - Protein encoded by GhRD22-like cDNA (clone OCF010D8).
SEQ ID NO:15 - Partial protein encoded by GhAsp cDNA (clone OCF008G9).
SEQ ID NO:16 - Partial protein encoded by cDNA clone CHX007D1.
SEQ ID NO:17 - GhHD1 cDNA (clone ON033M7).
SEQ ID NO:18 - Coding region of GhHD1 cDNA (clone ON033M7).
SEQ ID NO:19 - GhMyb25 cDNA (clone ON035F4).
SEQ ID NO:20 - Coding region for GhMyb25 cDNA (clone ON035F4).
SEQ ID NO:21 - GhCycD3;1 cDNA (clone OCF07F4).
SEQ ID NO:22 - Coding region of GhCycD3;1 cDNA (clone OCF07F4).
SEQ ID NO:23 - GhFaE1 cDNA (clone ON035N9) (entire clone coding region).
SEQ ID NO:24 - GhFU1 cDNA (clone ON035C9).
SEQ ID NO:25 - Coding region of GhFU1 cDNA (clone ON035C9).
SEQ ID NO:26 - GhFU2 cDNA (clone ON005F1).
SEQ ID NO:27 - Coding region of GhFU2 cDNA (clone ON005F1).
SEQ ID NO:28 - α-expansin like clone Pfs14x.
SEQ ID NO:29 - Coding region of α-expansin like clone Pfs14x.
SEQ ID NO:30 - GhTMTP cDNA (clone CHX015K18).
SEQ ID NO:31 - Coding region for GhTMTP cDNA (clone CHX015K18).
SEQ ID NO:32 - Ghsus cDNA (clone CHX002C10).
SEQ ID NO:33 - Coding region of Ghsus cDNA (clone CHX002C10).
SEQ ID NO:34 - GhLTP cDNA (clone ON033M19).
SEQ ID NO:35 - Coding region for GhLTP cDNA (clone ON033M19).
SEQ ID NO:36 - GhLTP2 cDNA (clone OCF010D8).
SEQ ID NO:37 - Coding region for GhLTP2 cDNA (clone OCF010D8).
SEQ ID NO:38 - GhMyb25-like cDNA (clone ON038N8) (entire clone coding region).
SEQ ID NO:39 - GhRD22 cDNA (clone OCF005C10).
SEQ ID NO:40 - Coding region for GhRD22 cDNA (clone OCF005C10).
SEQ ID NO:41 - GhRD22-like cDNA (clone OCF010D8).

SEQ ID NO:42 - Coding region for GhRD22-like cDNA (clone OCF010D8).
SEQ ID NO:43 - GhAsp cDNA (clone OCF008G9).
SEQ ID NO:44 - Coding region for GhAsp cDNA (clone OCF008G9).
SEQ ID NO:45 - cDNA clone CHX007D1 (entire clone coding region).
SEQ ID NO:46 - Cotton EST BE052193.
SEQ ID NO:47 - Cotton EST BQ412597.
SEQ ID NO:48 - Cotton EST AI731943.
SEQ ID NO:49 - Cotton EST BG442467.
SEQ ID NO:50 - Cotton EST BQ403714.
SEQ ID NO:51 - Cotton EST BG443329.
SEQ ID NO:52 - Cotton EST BF275177.
SEQ ID NO:53 - Cotton EST BQ410140.
SEQ ID NO:54 - Cotton EST CA993037.
SEQ ID NO:55 - Cotton EST BG441493.
SEQ ID NO:56 - Cotton EST BQ402375.
SEQ ID NO:57 - Cotton EST BQ413582.
SEQ ID NO's:58 to 64 - Stem loop sequences of dsRNA molecules.
SEQ ID NO's:65 to 72 - Oligonucleotide primers.

DETAILED DESCRIPTION OF THE INVENTION

General Techniques and Definitions

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, plant physiology and biochemistry, immunohistochemistry, protein chemistry, and biochemistry).

Unless otherwise indicated, the recombinant protein, cell culture and others methods utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al., (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan et al., (editors) Current Protocols

in Immunology, John Wiley & Sons (including all updates until present), and are incorporated herein by reference.

As used herein, the term "gene" is to be taken in its broadest context and includes the deoxyribonucleotide sequences comprising the protein coding region of a structural gene and including sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb on either end such that the gene corresponds to the length of the full-length mRNA. The sequences which are located 5' of the coding region and which are present on the mRNA are referred to as 5' non-translated sequences. The sequences which are located 3' or downstream of the coding region and which are present on the mRNA are referred to as 3' non-translated sequences; these sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region which may be interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene which are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide. The term "gene" includes a synthetic or fusion molecule encoding all or part of the proteins of the invention described herein and a complementary nucleotide sequence to any one of the above.

A "polymorphism" as used herein denotes a variation in the nucleotide sequence between genes of the invention, of different species, cultivars, strains or individuals of a plant. A "polymorphic position" is a preselected nucleotide position within the sequence of the gene. In some cases, genetic polymorphisms are reflected by an amino acid sequence variation, and thus a polymorphic position can result in location of a polymorphism in the amino acid sequence at a predetermined position in the sequence of a polypeptide. Typical polymorphisms are deletions, insertions or substitutions. These can involve a single nucleotide (single nucleotide polymorphism or SNP) or two or more nucleotides.

A "deletion," as used herein, refers to a change in either amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent.

An "insertion" or "addition," as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively.

A "substitution," as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

By "linked" or "genetically linked" it is meant that a marker locus and a second locus are sufficiently close on a chromosome that they will be inherited together in more than 50% of meioses, e.g., not randomly. Thus, the percent of recombination observed between the loci per generation (centimorgans (cM)), will be less than 50. In particular embodiments of the invention, genetically linked loci may be 45, 35, 25, 15, 10, 5, 4, 3, 2, or 1 or less cM apart on a chromosome. Preferably, the markers are less than 5 cM apart and most preferably about 0 cM apart.

The term "fibre" refers to plant cell types that share in common the features of having an elongated shape and abundant cellulose in thick cell walls, usually, but not always, described as secondary walls. Such walls may or may not be lignified, and the protoplast of such cells may or may not remain alive at maturity. Here the term "fibre" is used in its most inclusive sense, for example including: (a) thick-walled conducting and non-conducting cells of the xylem; (b) fibres of extraxylary origin, including those from ovary including the outer integument of the ovary, phloem, bark, ground tissue, and epidermis; and (c) fibres from stems, leaves, roots, seeds, and flowers or inflorescences. Such fibres have many industrial uses, for example in textiles, paper, sacking and boxing material, cordage, brushes and brooms, filling and stuffing, caulking, reinforcement of other materials, and manufacture of cellulose derivatives. In some industries, the term "fibre" is usually inclusive of thick-walled conducting cells such as vessels and tracheids and to fibrillar aggregates of many individual fibre cells. In a preferred embodiment, cotton fibre refers to the lint produced from the cotton boll (seed capsule) that is produced commercially. Cotton fibre also includes the short (about several mm) fibres sometimes referred to as "fuzz fibres". Preferred fibre producing plants include, but are not limited to, cotton (such as *Gossypium arboreum*, *Gossypium herbaceum*, *Gossypium barbadense* and *Gossypium hirsutum*), silk cotton tree (Kapok, Ceiba pentandra), desert willow, creosote bush, winterfal, balsa, ramie, kenaf, hemp (Cannabis sativa), roselle, jute, sisal abaca, flax, and horticultural plants such as grape, peach, pear, and apple.

As used herein, a "wild-type plant" is a plant that has not been altered by a method of the invention and/or does not comprise a transgene of the invention. When performing a method of the invention for altering fibre initiation and/or elongation, the manipulated plant is compared to a non-manipulated ("wild-type") member of the same species to determine the impact of the manipulation on fibre initiation and/or elongation.

In one embodiment, the term "altering fibre initiation and/or elongation" refers to increasing the number and/or size of the fibres. In another embodiment, the term "altering fibre initiation and/or elongation" refers to decreasing the number and/or size of the fibres. In a further embodiment, the term "altering fibre initiation and/or elongation" refers to modifying the timing of fibre initiation and/or elongation during

development of the plant, for example to promote earlier or delayed initiation, or to regulate the synchrony of fibre initiation.

Detection of impaired genes and/or gene expression levels

Any molecular biological technique known in the art which is capable of detecting a polymorphism/mutation/genetic variation or differential gene expression can be used in the methods of the present invention. Such methods include, but are not limited to, the use of nucleic acid amplification, nucleic acid sequencing, nucleic acid hybridization with suitably labeled probes, single-strand conformational analysis (SSCA), denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis (HET), chemical cleavage analysis (CCM), catalytic nucleic acid cleavage, or a combination thereof (see, for example, Lemieux, 2000). The invention also includes the use of molecular marker techniques to detect polymorphisms closely linked to genes of the invention. Such methods include the detection or analysis of restriction fragment length polymorphisms (RFLP), RAPD, amplified fragment length polymorphisms (AFLP) and microsatellite (simple sequence repeat, SSR) polymorphisms. The closely linked markers can be obtained readily by methods well known in the art, such as Bulked Segregant Analysis.

The "polymerase chain reaction" ("PCR") is a reaction in which replicate copies are made of a target polynucleotide using a "pair of primers" or "set of primers" consisting of "upstream" and a "downstream" primer, and a catalyst of polymerization, such as a DNA polymerase, and typically a thermally-stable polymerase enzyme. Methods for PCR are known in the art, and are taught, for example, in "PCR" (Ed. M.J. McPherson and S.G Moller (2000) BIOS Scientific Publishers Ltd, Oxford). PCR can be performed on cDNA obtained from reverse transcribing mRNA isolated from plant cells expressing, or that should be expressing, a gene of the invention. However, it will generally be easier if PCR is performed on genomic DNA isolated from a plant.

A primer is an oligonucleotide, usually of about 20 nucleotides long, with a minimum of about 15 and a maximum of about 50 nucleotides, that is capable of hybridising in a sequence specific fashion to the target sequence and being extended during the PCR. Amplicons or PCR products or PCR fragments or amplification products are extension products that comprise the primer and the newly synthesized copies of the target sequences. Multiplex PCR systems contain multiple sets of primers that result in simultaneous production of more than one amplicon.. Primers may be perfectly matched to the target sequence or they may contain internal mismatched bases that can result in the induction of restriction enzyme or catalytic nucleic acid recognition/cleavage sites in specific target sequences. Primers may also contain additional sequences and/or modified or labelled nucleotides to facilitate capture or

detection of amplicons. Repeated cycles of heat denaturation of the DNA, annealing of primers to their complementary sequences and extension of the annealed primers with polymerase result in exponential amplification of the target sequence. The terms target or target sequence or template refer to nucleic acid sequences which are amplified.

Methods for direct sequencing of nucleotide sequences are well known to those skilled in the art and can be found for example in Ausubel et al., eds., Short Protocols in Molecular Biology, 3rd ed., Wiley, (1995) and Sambrook et al., Molecular Cloning, 2nd ed., Chap. 13, Cold Spring Harbor Laboratory Press, (1989). Sequencing can be carried out by any suitable method, for example, dideoxy sequencing, chemical sequencing or variations thereof. Direct sequencing has the advantage of determining variation in any base pair of a particular sequence.

Hybridization based detection systems include, but are not limited to, the TaqMan assay and molecular beacons. The TaqMan assay (US 5,962,233) uses allele specific (ASO) probes with a donor dye on one end and an acceptor dye on the other end such that the dye pair interact via fluorescence resonance energy transfer (FRET). A target sequence is amplified by PCR modified to include the addition of the labeled ASO probe. The PCR conditions are adjusted so that a single nucleotide difference will effect binding of the probe. Due to the 5' nuclease activity of the Taq polymerase enzyme, a perfectly complementary probe is cleaved during PCR while a probe with a single mismatched base is not cleaved. Cleavage of the probe dissociates the donor dye from the quenching acceptor dye, greatly increasing the donor fluorescence.

An alternative to the TaqMan assay is the molecular beacon assay (US 5,925,517). In the molecular beacon assay, the ASO probes contain complementary sequences flanking the target specific species so that a hairpin structure is formed. The loop of the hairpin is complimentary to the target sequence while each arm of the hairpin contains either donor or acceptor dyes. When not hybridized to a donor sequence, the hairpin structure brings the donor and acceptor dye close together thereby extinguishing the donor fluorescence. When hybridized to the specific target sequence, however, the donor and acceptor dyes are separated with an increase in fluorescence of up to 900 fold. Molecular beacons can be used in conjunction with amplification of the target sequence by PCR and provide a method for real time detection of the presence of target sequences or can be used after amplification.

Marker assisted selection is a well recognised method of selecting for heterozygous plants required when backcrossing with a recurrent parent in a classical breeding program. The population of plants in each backcross generation will be heterozygous for the gene of interest, normally present in a 1:1 ratio in a backcross population, and the molecular marker can be used to distinguish the two alleles. By extracting DNA from, for example, young leaves or shoots and testing with a specific

marker for the introgressed desirable trait, early selection of plants for further backcrossing is made whilst energy and resources are concentrated on fewer plants.

Polypeptides

By "substantially purified polypeptide" we mean a polypeptide that has been at least partially separated from the lipids, nucleic acids, other polypeptides, and other contaminating molecules with which it is associated in its native state. Preferably, the substantially purified polypeptide is at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated. Furthermore, the term "polypeptide" is used interchangeably herein with the term "protein".

The % identity of a polypeptide is determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. Unless stated otherwise, the query sequence is at least 15 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 15 amino acids. More preferably, the query sequence is at least 50 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 50 amino acids. Even more preferably, the query sequence is at least 100 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 100 amino acids.

Amino acid sequence mutants of the polypeptides of the present invention can be prepared by introducing appropriate nucleotide changes into a nucleic acid of the present invention, or by *in vitro* synthesis of the desired polypeptide. Such mutants include, for example, deletions, insertions or substitutions of residues within the amino acid sequence. A combination of deletion, insertion and substitution can be made to arrive at the final construct, provided that the final protein product possesses the desired characteristics.

In designing amino acid sequence mutants, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting other residues adjacent to the located site.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Substitution mutants have at least one amino acid residue in the polypeptide molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active or binding site(s). Other sites of interest are those in which particular residues obtained from

various strains or species are identical. These positions may be important for biological activity. These sites, especially those falling within a sequence of at least three other identically conserved sites, are preferably substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1.

TABLE 1. Exemplary substitutions.

Original Residue	**Exemplary Substitutions**
Ala (A)	val; leu; ile; gly
Arg (R)	lys
Asn (N)	gln; his
Asp (D)	glu
Cys (C)	ser
Gln (Q)	asn; his
Glu (E)	asp
Gly (G)	pro, ala
His (H)	asn; gln
Ile (I)	leu; val; ala
Leu (L)	ile; val; met; ala; phe
Lys (K)	arg
Met (M)	leu; phe
Phe (F)	leu; val; ala
Pro (P)	gly
Ser (S)	thr
Thr (T)	ser
Trp (W)	tyr
Tyr (Y)	trp; phe
Val (V)	ile; leu; met; phe, ala

Furthermore, if desired, unnatural amino acids or chemical amino acid analogues can be introduced as a substitution or addition into the polypeptides of the present invention. Such amino acids include, but are not limited to, the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, 2-aminobutyric acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline,

homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogues in general.

Also included within the scope of the invention are polypeptides of the present invention which are differentially modified during or after synthesis, e.g., by biotinylation, benzylation, glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. These modifications may serve to increase the stability and/or bioactivity of the polypeptide of the invention.

Polypeptides of the present invention can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an isolated polypeptide of the present invention is produced by culturing a cell capable of expressing the polypeptide under conditions effective to produce the polypeptide, and recovering the polypeptide. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce a polypeptide of the present invention. Such medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art.

In a preferred embodiment, the polypeptides of the invention are transcription factors, regulatory proteins, or proteins that regulate the cell-cycle in the fibre producing plant. The transcription factors may be Myb transcription factors or homeodomain containing transcription factors, which are classes well known in the art.

Polynucleotides

By an "isolated polynucleotide", including DNA, RNA, or a combination of these, single or double stranded, in the sense or antisense orientation or a combination of both, dsRNA or otherwise, we mean a polynucleotide which is at least partially separated from the polynucleotide sequences with which it is associated or linked in its native state. Preferably, the isolated polynucleotide is at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which

they are naturally associated. Furthermore, the term "polynucleotide" is used interchangeably herein with the term "nucleic acid molecule".

The % identity of a polynucleotide is determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. Unless stated otherwise, the query sequence is at least 45 nucleotides in length, and the GAP analysis aligns the two sequences over a region of at least 45 nucleotides. Preferably, the query sequence is at least 150 nucleotides in length, and the GAP analysis aligns the two sequences over a region of at least 150 nucleotides. More preferably, the query sequence is at least 300 nucleotides in length and the GAP analysis aligns the two sequences over a region of at least 300 nucleotides.

Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for the formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention. Preferably, the oligonucleotides are at least 15 nucleotides, more preferably at least 18 nucleotides, more preferably at least 19 nucleotides, more preferably at least 20 nucleotides, even more preferably at least 25 nucleotides in length. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules or as agents to modify fibre initiation and/or elongation (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). Oligonucleotide of the present invention used as a probe are typically conjugated with a label such as a radioisotope, an enzyme, biotin, a fluorescent molecule or a chemiluminescent molecule.

Polynucleotides and oligonucleotides of the present invention include those which hybridize under stringent conditions to a sequence provided as SEQ ID NO's: 17 to 45. As used herein, stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% $NaDodSO_4$ at 50^0C; (2) employ during hybridisation a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42^0C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS and 10% dextran sulfate at 42^0C in 0.2 x SSC and 0.1% SDS.

Polynucleotides of the present invention may possess, when compared to naturally occurring molecules, one or more mutations which are deletions, insertions, or substitutions of nucleotide residues. Mutants can be either naturally occurring (that is to

say, isolated from a natural source) or synthetic (for example, by performing site-directed mutagenesis on the nucleic acid).

Antisense Polynucleotides

The term "antisense nucleic acid" shall be taken to mean DNA or RNA, or combination thereof, molecule that is complementary to at least a portion of a specific mRNA molecule of the invention and capable of interfering with a post-transcriptional event such as mRNA translation. . The use of antisense methods is well known in the art (see for example, G. Hartmann and S. Endres, Manual of Antisense Methodology, Kluwer (1999)). The use of antisense techniques in plants has been reviewed by Bourque (1995) and Senior (1998). Bourque lists a large number of examples of how antisense sequences have been utilized in plant systems as a method of gene inactivation. She also states that attaining 100% inhibition of any enzyme activity may not be necessary as partial inhibition will more than likely result in measurable change in the system. Senior (1998) states that antisense methods are now a very well established technique for manipulating gene expression.

Antisense molecules may include sequences that correspond to the structural genes or for sequences that effect control over the gene expression or splicing event. For example, the antisense sequence may correspond to the targeted coding region of the genes of the invention, or the 5'-untranslated region (UTR) or the 3'-UTR or combination of these. It may be complementary in part to intron sequences, which may be spliced out during or after transcription, preferably only to exon sequences of the target gene. In view of the generally greater divergence of the UTRs, targeting these regions provides greater specificity of gene inhibition. The length of the antisense sequence should be at least 19 contiguous nucleotides, preferably at least 50 nucleotides, and more preferably at least 100, 200, 500 or 1000 nucleotides. The full-length sequence complementary to the entire gene transcript may be used. The length is most preferably 100-2000 nucleotides. The degree of homology of the antisense sequence to the targeted transcript should be at least 85%, preferably at least 90% and more preferably 95-100%. The antisense RNA molecule may of course comprise unrelated sequences which may function to stabilize the molecule.

Catalytic Polynucleotides

The term catalytic polynucleotide/nucleic acid refers to a DNA molecule or DNA-containing molecule (also known in the art as a "deoxyribozyme") or an RNA or RNA-containing molecule (also known as a "ribozyme") which specifically recognizes a distinct substrate and catalyzes the chemical modification of this substrate. The nucleic acid bases in the catalytic nucleic acid can be bases A, C, G, T (and U for RNA).

Typically, the catalytic nucleic acid contains an antisense sequence for specific recognition of a target nucleic acid, and a nucleic acid cleaving enzymatic activity (also referred to herein as the "catalytic domain"). The types of ribozymes that are particularly useful in this invention are the hammerhead ribozyme (Haseloff and Gerlach, 1988, Perriman et al., 1992) and the hairpin ribozyme (Shippy et al., 1999).

The ribozymes of this invention and DNA encoding the ribozymes can be chemically synthesized using methods well known in the art. The ribozymes can also be prepared from a DNA molecule (that upon transcription, yields an RNA molecule) operably linked to an RNA polymerase promoter, e.g., the promoter for T7 RNA polymerase or SP6 RNA polymerase. Accordingly, also provided by this invention is a nucleic acid molecule, i.e., DNA or cDNA, coding for the ribozymes of this invention. When the vector also contains an RNA polymerase promoter operably linked to the DNA molecule, the ribozyme can be produced *in vitro* upon incubation with RNA polymerase and nucleotides. In a separate embodiment, the DNA can be inserted into an expression cassette or transcription cassette. After synthesis, the RNA molecule can be modified by ligation to a DNA molecule having the ability to stabilize the ribozyme and make it resistant to RNase.

RNA interference

RNA interference (RNAi) is particularly useful for specifically inhibiting the production of a particular protein. Although not wishing to be limited by theory, Waterhouse et al. (1998) have provided a model for the mechanism by which dsRNA can be used to reduce protein production. . This technology relies on the presence of dsRNA molecules that contain a sequence that is essentially identical to the mRNA of the gene of interest or part thereof, in this case an mRNA encoding a polypeptide according to the invention. Conveniently, the dsRNA can be produced from a single promoter in a recombinant vector or host cell, where the sense and anti-sense sequences are flanked by an unrelated sequence which enables the sense and anti-sense sequences to hybridize to form the dsRNA molecule with the unrelated sequence forming a loop structure. The design and production of suitable dsRNA molecules for the present invention is well within the capacity of a person skilled in the art, particularly considering Waterhouse et al. (1998), Smith et al. (2000), WO 99/32619, WO 99/53050, WO 99/49029, and WO 01/34815. In this method a DNA is introduced that directs the synthesis of an at least partly double stranded RNA product(s) with homology to the target gene to be inactivated. The DNA therefore comprises both sense and antisense sequences that, when transcribed into RNA, can hybridize to form the double-stranded RNA region. In a preferred embodiment, the sense and antisense sequences are separated by a spacer region that comprises an intron which, when transcribed into

RNA, is spliced out. This arrangement has been shown to result in a higher efficiency of gene silencing. The double-stranded region may comprise one or two RNA molecules, transcribed from either one DNA region or two. The presence of the double stranded molecule is thought to trigger a response from an endogenous plant system that destroys both the double stranded RNA and also the homologous RNA transcript from the target plant gene, efficiently reducing or eliminating the activity of the target gene. The length of the sense and antisense sequences that hybridise should each be at least 19 contiguous nucleotides, preferably at least 30 or 50 nucleotides, and more preferably at least 100, 200, 500 or 1000 nucleotides. The full-length sequence corresponding to the entire gene transcript may be used. The lengths are most preferably 100-2000 nucleotides. The degree of homology of the sense and antisense sequences to the targeted transcript should be at least 85%, preferably at least 90% and more preferably 95-100%. The RNA molecule may of course comprise unrelated sequences which may function to stabilize the molecule. The RNA molecule may be expressed under the control of a RNA polymerase II or RNA polymerase III promoter. Examples of the latter include tRNA or snRNA promoters.

Preferred small interfering RNA ('siRNA") molecules comprise a nucleotide sequence that is identical to about 19-21 contiguous nucleotides of the target mRNA. Preferably, the target mRNA sequence commences with the dinucleotide AA, comprises a GC-content of about 30-70% (preferably, 30-60%, more preferably 40-60% and more preferably about 45%-55%), and does not have a high percentage identity to any nucleotide sequence other than the target in the genome of the plant (preferably cotton) in which it is to be introduced, e.g., as determined by standard BLAST search.

Preferred loop ("single stranded") sequences are selected from, but not limited to, the group consisting of:

(i) CCC (SEQ ID NO:58);
(ii) UUCG (SEQ ID NO:59);
(iii) CCACC (SEQ ID NO:60);
(iv) CUCGAG (SEQ ID NO:61);
(v) AAGCUU (SEQ ID NO:62);
(vi) CCACACC (SEQ ID NO:63); and
(vii) UUCAAGAGA (SEQ ID NO:64).

Another molecular biological approach that may be used is co-suppression. The mechanism of co-suppression is not well understood but is thought to involve post-transcriptional gene silencing (PTGS) and in that regard may be very similar to many examples of antisense suppression. It involves introducing an extra copy of a gene or a fragment thereof into a plant in the sense orientation with respect to a promoter for its expression. The size of the sense fragment, its correspondence to target gene regions,

and its degree of homology to the target gene are as for the antisense sequences described above. In some instances the additional copy of the gene sequence interferes with the expression of the target plant gene. Reference is made to Patent specification WO 97/20936 and European patent specification 0465572 for methods of implementing co-suppression approaches. The antisense, cosuppression or double stranded RNA molecules may also comprise a largely double-stranded RNA region, preferably comprising a nuclear localization signal, as described in PCT/AU03/00292. In a preferred embodiment, the largely double-stranded region is derived from a PSTVd type viroid or comprises at least 35 CUG trinucleotide repeats.

Transgenic Plants

The term "plant" refers to whole plants, plant organs (e.g. leaves, stems roots, etc), seeds, plant cells and the like. Plants contemplated for use in the practice of the present invention include both monocotyledons and dicotyledons. Preferably, the plant is a horticultural plant or cotton.

The term "cotton" as used herein includes any species of the genus *Gossypium* which is used for commercial fibre production, preferably *G. hirsutum* or *G. barbadense*.

Transgenic plants, as defined in the context of the present invention include plants (as well as parts and cells of said plants) and their progeny which have been genetically modified using recombinant techniques. This would generally be to either i) cause or enhance production of at least one protein of the present invention in the desired plant or plant organ, or ii) disrupt the production and/or activity of a polypeptide of the present invention. Transformed plants contain genetic material that they did not contain prior to the transformation. The genetic material is preferably stably integrated into the genome of the plant. The introduced genetic material may comprise sequences that naturally occur in the same species but in a rearranged order or in a different arrangement of elements, for example an antisense sequence. Such plants are included herein in "transgenic plants". A "non-transgenic plant" is one which has not been genetically modified with the introduction of genetic material by recombinant DNA techniques.

Several techniques exist for introducing foreign genetic material into a plant cell. Such techniques include acceleration of genetic material coated onto microparticles directly into cells (see, for example, US 4,945,050 and US 5,141,131). Plants may be transformed using *Agrobacterium* technology (see, for example, US 5,177,010, US 5,104,310, US 5,004,863, US 5,159,135). Electroporation technology has also been used to transform plants (see, for example, WO 87/06614, US 5,472,869, 5,384,253, WO 92/09696 and WO 93/21335). In addition to numerous technologies for

transforming plants, the type of tissue which is contacted with the foreign genes may vary as well. Such tissue would include but would not be limited to embryogenic tissue, callus tissue type I and II, hypocotyl, meristem, and the like. Almost all plant tissues may be transformed during development and/or differentiation using appropriate techniques described herein.

A particularly preferred method of producing a transgenic cotton plant is by Agrobacterium-mediated transformation of cotyledons, followed by the induction of callus formation, and the subsequent induction of embryogenic callus, and regeneration into plants.

A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., Cloning Vectors: A Laboratory Manual, 1985, supp. 1987; Weissbach and Weissbach, Methods for Plant Molecular Biology, Academic Press, 1989; and Gelvin et al., Plant Molecular Biology Manual, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

Examples of plant promoters include, but are not limited to ribulose-1,6-bisphosphate carboxylase small subunit, beta-conglycinin promoter, phaseolin promoter, high molecular weight glutenin (HMW-GS) promoters, starch biosynthetic gene promoters, ADH promoter, heat-shock promoters and tissue specific promoters. Promoters may also contain certain enhancer sequence elements that may improve the transcription efficiency. Typical enhancers include but are not limited to Adh-intron 1 and Adh-intron 6.

Constitutive promoters direct continuous gene expression in all cells types and at all times (e.g., actin, ubiquitin, CaMV 35S). Tissue specific promoters are responsible for gene expression in specific cell or tissue types, such as the leaves or seeds (e.g., zein, oleosin, napin, ACP, globulin and the like) and these promoters may also be used. Promoters may also be active during a certain stage of the plants' development as well as active in plant tissues and organs. Examples of such promoters include but are not limited to pollen-specific, embryo specific, corn silk specific, cotton fibre specific, root specific, seed endosperm specific promoters and the like.

In a particularly preferred embodiment, the promoter directs expression around anthesis which is when fibre initiation and elongation occur. Thus, it is preferred that

the promoter is an ovule or fibre specific promoter. Examples include promoters described in U.S. 5,495,070, U.S. 5,608,148 and U.S. 5,602,321.

Under certain circumstances it may be desirable to use an inducible promoter. An inducible promoter is responsible for expression of genes in response to a specific signal, such as: physical stimulus (heat shock genes); light (RUBP carboxylase); hormone (Em); metabolites; and stress. Other desirable transcription and translation elements that function in plants may be used.

In addition to plant promoters, promoters from a variety of sources can be used efficiently in plant cells to express foreign genes. For example, promoters of bacterial origin, such as the octopine synthase promoter, the nopaline synthase promoter, the mannopine synthase promoter; promoters of viral origin, such as the cauliflower mosaic virus (35S and 19S) and the like may be used.

Vectors

One embodiment of the present invention includes a recombinant vector, which includes at least one isolated polynucleotide molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid.

One type of recombinant vector comprises a nucleic acid molecule of the present invention operatively linked to an expression vector. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, endoparasite, arthropod, other animal, and plant cells. Preferred expression vectors of the present invention can direct gene expression in plant cells.

In particular, expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention.

In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art.

Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained.

<u>Host cells</u>

Suitable host cells to transform include any cell that can be transformed with a polynucleotide of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), parasite, arthropod, animal and plant cells. Preferred host cells are plant cells, in particular cotton cells. In a preferred embodiment, the cells are ovule cells such as the cells of the outer integument of cotton ovules.

<u>Antibodies</u>

The invention also provides monoclonal or polyclonal antibodies to polypeptides of the invention or fragments thereof. Thus, the present invention further provides a

process for the production of monoclonal or polyclonal antibodies to polypeptides of the invention.

The term "binds specifically" refers to the ability of the antibody to bind to proteins of the present invention but not other proteins obtained of the plant.

As used herein, the term "epitope" refers to a region of a protein of the invention which is bound by the antibody. An epitope can be administered to an animal to generate antibodies against the epitope, however, antibodies of the present invention preferably specifically bind the epitope region in the context of the entire protein.

If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunised with an immunogenic polypeptide. Serum from the immunised animal is collected and treated according to known procedures. If serum containing polyclonal antibodies contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art. In order that such antibodies may be made, the invention also provides polypeptides of the invention or fragments thereof haptenised to another polypeptide for use as immunogens in animals or humans.

Monoclonal antibodies directed against polypeptides of the invention can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Panels of monoclonal antibodies produced can be screened for various properties; i.e., for isotype and epitope affinity.

An alternative technique involves screening phage display libraries where, for example the phage express scFv fragments on the surface of their coat with a large variety of complementarity determining regions (CDRs). This technique is well known in the art.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments of whole antibodies which retain their binding activity for a target antigen. Such fragments include Fv, F(ab') and $F(ab')_2$ fragments, as well as single chain antibodies (scFv). Furthermore, the antibodies and fragments thereof may be humanised antibodies, for example as described in EP-A-239400.

Antibodies of the invention may be bound to a solid support and/or packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like.

Preferably, antibodies of the present invention are detectably labeled. Exemplary detectable labels that allow for direct measurement of antibody binding include

radiolabels, fluorophores, dyes, magnetic beads, chemiluminescers, colloidal particles, and the like. Examples of labels which permit indirect measurement of binding include enzymes where the substrate may provide for a coloured or fluorescent product. Additional exemplary detectable labels include covalently bound enzymes capable of providing a detectable product signal after addition of suitable substrate. Examples of suitable enzymes for use in conjugates include horseradish peroxidase, alkaline phosphatase, malate dehydrogenase and the like. Where not commercially available, such antibody-enzyme conjugates are readily produced by techniques known to those skilled in the art. Further exemplary detectable labels include biotin, which binds with high affinity to avidin or streptavidin; fluorochromes (e.g., phycobiliproteins, phycoerythrin and allophycocyanins; fluorescein and Texas red), which can be used with a fluorescence activated cell sorter; haptens; and the like. Preferably, the detectable label allows for direct measurement in a plate luminometer, e.g., biotin. Such labeled antibodies can be used in techniques known in the art to detect proteins of the invention.

<u>Assessing Fibre Properties</u>

Fibres produced from plants of the invention are compared to control fibres (e.g., fibres from wild-type plants or plants transformed with marker nucleic acids) to determine the extent of modulation of fibre properties. Modulation of fibre properties, such as fibre number, length, strength, or fineness, is achieved when the percent difference in these fibre properties of the plants of the invention and control plants is at least about 10%, preferably at least about 20%, most preferably at least about 30%.

Several parameters can be measured to compare the properties or quality of fibres produced from plants of the invention to wild-type plants. These include: 1) fibre number, 2) fibre length; 3) fibre strength; and 4) fineness of fibres.

A number of methods are known in the art to measure these parameters, such as described in U.S. 5,495,070. For example, instruments such as a fibrograph and HVI (high volume instrumentation) systems can be used to measure the length of fibres. The HVI systems can also be used to measure fibre strength. Fibre strength generally refers to the force required to break a bundle of fibres or a single fibre. In HVI testing, the breaking force is expressed in terms of "grams force per tex unit." This is the force required to break a bundle of fibres that is one tex unit in size. In addition, fineness of fibres can be measured, e.g., from a porous air flow test. In a porous air flow test, a weighed sample of fibres is compressed to a given volume and controlled air flow is passed through the sample. The resistance to the air flow is read as micronaire units. More specifically, the micronaire value is a measurement of cotton fibre quality that is a reflection of both fineness and maturity; low values indicate fine and/or immature fibre;

high values indicate coarse and/or mature fibres. These values are determined according to standard techniques by measuring the resistance offered by a plug of cotton to airflow (supra) that is influenced by a combination of fineness and maturity. Fineness is the outside diameter of the fibre that is measured in mTex (Millitex or mg/km). Maturity is the degree of wall thickening of the fibre. Short fibre count (w) % refers to the percentage of short fibre weight; short fibre count (n) % refers to the percentage of short fibre yield. Immature fibre count refers to the number of immature fibres, i.e., fibres in which the thickening of the fibre wall is appreciably less than normal. An increase in fibre yield (fibre weight/seed) can be measured by using the Advanced Fibre Information System (AFIS). Using these and other methods known in the art, one of skill can readily determine the extent of modulation of fibre characteristics, quality and/or yield plants produced by the methods of the invention.

EXAMPLES

Example 1 - Comparison of fibre initials development in wild-type and lintless mutants of cotton.

To identify genes that were specific to fibre initiation, genes were identified that were differentially expressed in early stage fertilised ovules of wild-type and lintless mutants of cotton that produce little if any fibres. The cotton lines (*Gossypium hirsutum L.*) used included two wild type cotton lines: Deltapine 16 (DP16) and Xu-142, and 6 lintless lines: Lintless 1A, Lintless 4A, Lintless 5B, Lintless 53, SL1-7-1 and *fl*. The 1A, 4A, 5B, 53 lines were obtained from the Queensland Department of Primary Industry Tropical Crops and Pastures Germplasm Collection and were originally selections from a linted cultivar B1278 isolated by Dr Alistair Low (unpublished, CSIRO Irrigation Research, Griffith, NSW). SL1-7-1 was obtained from USDA-ARS (College Station, Texas, USA). The *Fuzzless-lintless, fl*, isolated from Xu-142 background, and Xu-142 were provided by Prof. Xiao-Ya Chen (Institute of Plant Physiology, Chinese Academy of Science, Shanghai, China). All the cotton lines were grown in a glasshouse with temperature of 30 °C /22 °C (day/night). Ovules were always collected at a similar time each day (1-3 pm) and samples to be compared on a microarray were only used when they were collected on the same date and from the same glasshouse to minimise between time or location variability.

The mutants were of varying provenance but it is not known whether they are allelic. The four lines Lintless 1A, Lintless 4A, Lintless 5B, Lintless 53 were originally separate selections from a fully linted cultivar B1278 as spontaneous mutants that showed low but differing levels of lint production isolated as part of a breeding program to produce a cultivar with high quality seed oil and protein but with only a small amount of lint to retain the seeds in the boll capsule (Alistair Low, unpublished). Genetic

complementation testing has not yet been carried out. SL 1-7-1 (Mississipi Obsolete Collection Number 0504) was also a naturally occurring variant (Turley and Ferguson, 1996) that produced less lint than the most lintless of the B1278 selections. All the mutant lines, but particularly 5B which had the most lint of any of the lines, show a variable, but low level of leakiness and this may be a result of environmental or physiological influences on lint production. At maturity the seeds of all of the mutant lines exhibit a fuzz-less phenotype (ie they have a completely naked seed and lack the short fuzz fibres covering the ovules of most Upland cotton varieties, including B1278). The growth rate and general vegetative and floral development of the mutants were similar to the wild type except that lintless 4A was slightly slimmer, and taller (about 20%) than the Deltapine 16 (DP16) variety used as the wildtype, whereas SL 1-7-1 (SL) exhibited higher levels of red anthocyanin pigments in all parts of the plant. All lines produced normal amounts of trichomes on their stems and leaves.

Scanning electron microscopy was used to examine the fibre development of the 5 lintless cotton mutants (Lintless 1A, 4A, 5B, 53 and SL) at two days before anthesis (–2 dpa), the day of anthesis (0 dpa) and two days after anthesis (2 dpa). Cotton ovules were collected using the phyllotactic arrangement of cotton flowering nodes and size of cotton flower buds as indicator of the development stage as described by Hasenfratz et al., (1995). The collected ovules were observed using an Oxford CT 1500 cryotrans system attached to a JEOL 6400 scanning electron microscope as described by Craig and Beaton (1996). There was generally no obvious difference between the mutants and the wild type (DP16) at –2 dpa except that 4A ovules were covered with mucous-like substances. The ovule surfaces were flat and epidermal cells were interspersed with stomata. On the day of anthesis, a few fibre initials became visible on the mutant ovules but these were considerably fewer in number than on wild type ovules. The mucous-like substances covering 4A ovule disappeared at this stage. The mutant phenotype was best revealed at two days post-anthesis when the fibres have begun to elongate. At this time, all of the mutants had a much reduced number of fibre initials on the surface of ovules and those fibres that had developed were slower to elongate and less synchronized in their elongation than the fibre initials of the wild type. The degree of lintlessness varied among the mutants with some lines showing very few fibre initials (Lintless 4A, 1A and 53) and the others more (Lintless 5B and SL-1-7-1), but still considerably fewer than DP16. Early fibre growth was sensitive to environmental conditions demonstrated by the more rapid fibre growth of the wild type cotton grown in glasshouses with a temperature regime of 30°C/22°C (day/night) than in a glasshouse with temperature of 25°C/15°C.

Example 2 - Differential expression of genes in the mutant ovules compared to wild-type.

cDNA Library Construction

CHX cDNA library was constructed using cycloheximide treated ovules. Cotton flower buds of developmental stages of -3 dpa, -2 dpa, -1 dpa and 0 dpa were detached and pooled from glasshouse grown cotton plants and surface sterilized by dipping in 70% ethanol and flaming twice. The cotton ovules were dissected out under sterile conditions and cultured on 15 ml of cotton ovule culture medium (Beasley and Ting, 1973) supplemented with 5 μM IAA (indole acetic acid) and 1 μM GA in 100 ml glass flasks at 29 °C in the dark overnight and then treated with 10 μM cycloheximide for 4 hours under the same culture conditions. After the cycloheximide treatment, the ovules were rinsed with sterile water, stored in RNAlater solution (Ambion) at –20 °C. Total RNA was isolated using a method described by Wu et al. (2002). Purification of poly A^+ mRNA from total RNA was carried out using Qiagen Oligotex mRNA kit (Cat. No. 70042), following the manufacturer's protocol. *In vitro* translation was carried out to verify the bioactivity of the poly A^+ RNA (Wu et al. 2002). 5 μg poly A^+ mRNA from cycloheximide treated ovules was used for cDNA synthesis using a Life Technologies' Superscript Choice system (Cat. Series 18090) following the manufacturer's instructions. The first strand cDNA was synthesized using a mix of 1 μg Oligo(dT)12-18 primer and 50 ng of random hexamers. The *Eco*RI adapted cDNA was size-fractionated and cDNA longer than 500 bp was randomly cloned in λZipLox *EcoRI* arms (Life Technology Cat No. 15397-029). This library was comprised of 2 x 106 primary pfu with average insert size of 1.05 kb.

OCF cDNA library was developed from DP16 0 dpa ovules. The total RNA isolation and poly A^+ mRNA purification was the same as for CHX library construction. 5 μg poly A^+ mRNA was used for cDNA synthesis and cDNA library construction using a Life Technologies' Superscript lamda system (Cat. No. 19643-014) following the manufacturer's instructions. The first strand cDNA was synthesized using the *NotI* primer-adapter, in the presence of 1 μCi [α-^{32}P] dCTP. The *SalI* adapted and *NotI* digested cDNA was size-fractionated and the cDNA longer than 500 bp was directionally cloned in λZipLox *NotI* – *SalI* arms (Life Technology Cat No. 15397-029). This primary library was comprised of 5 x 10^5 pfu with average insert size of 0.9 kb.

For both CHX and OCF libraries, the cDNA could be recovered in the autonomously-replicating plasmid pZL1 using a *in vivo* excision protocol provided by Life Technology. Two bacterial strains (Life Technology) were used for the excision: DH10B (ZIP) for the preparation of double stranded plasmid DNA, and DH12S (ZIP) for the preparation of single stranded DNA by infection with helper phage M13K07 (NEW ENGLAND BioLabs).

Normalization of the OCF library

The OCF library was normalised using a method essentially as described by Bonaldo et al. (1996) with some modifications. Single-stranded plasmid DNA (ssDNA) was prepared from the excised OCF library using helper phage M13K07 (NEW ENGLAND BioLabs) and purified using the Bio-Gel HTP hydroxyapatite (HAP) column (Bio-Rad) according to Ali et al. (2000) with some modifications. The ssDNA was loaded on to a jacketed HAP column (Bio-Rad) at 60 °C and washed with 3 ml 10 mM Na-phosphate. The column was washed with 3ml 0.16M Na-phosphate buffer and the eluate was collected in small fractions (about) 200 μl) and OD_{260} was measured for each of the fractions. The fractions with the highest OD measurements were pooled and desalted using a Qiaquick PCR purification kit (Qiagen). The driver DNA was prepared from the same excised OCF library by SalI and NotI digestion of the double-stranded plasmid and the gel-separated cDNA fragments (smear on the gel) was purified using a Qiaquick gel extraction kit (Qiagen). Hybridization of driver with ssDNA tracer was performed in the presence of a 5'-blocking oligo (5'-CCCACGCGTCCG-3') (SEQ ID NO:71), and a 3'-blocking oligo (5'-AAAAAAGGGCGGCC-3') (SEQ ID NO:72). The hybridization mix comprised 0.5 M NaCl, 10 mM Tris (pH 8.0), 1 mM EDTA, 2 μg double-stranded driver DNA, 0.2 μg ssDNA, 10 μg of each of the blocking oligos, and 25 μl formamide in a total volume of 50 μl. The driver DNA was heat denatured by boiling for 5 min prior to mixing with the rest of the components and the whole hybridisation mix was heated to 65°C for 3 min and then incubated at 42 °C for 24 hours. The hybridisation mix was run through a HAP column under the same conditions as described for ssDNA purification to separate the ssDNA from the hybridised DNA. The conversion of purified ssDNA into double-stranded plasmid DNA and transformation of competent DH10B (Life Technology) was as described by Ali et al. (2000). The normalized library was designated the ON library consisting 10^5 primary transformants with average insert size of 0.9 kb.

PCR amplification of cDNA clones and microarray preparation

The cotton ovule cDNA microarray comprises a total of 10410 cDNA clones. Except for 52 clones encoding known cotton genes provided by colleagues, and 13 clones of negative controls (non-plant genes, intron sequences etc.), the rest of the clones were randomly picked from the CHX, OCF and ON cDNA libraries, including 5496 clones from CHX library, 1149 clones from OCF library and 3700 clones from the ON library.

All the anonymous cDNA clones from the ovule cDNA libraries were PCR amplified in 96 well PCR plates (AB gene). The PCR reaction contained of 2 mM $MgCl_2$, 0.2 mM each of the dNTPs, 0.2 μM each of the M13/pUC forward and reverse

primers, 1 unit Taq F2 DNA polymerase and 1x F2 buffer (BIOTECH International Limited) in a 50 µl reaction with 2 µl overnight cultured bacterial cells as template. A row of 12 samples from each PCR plate was verified by agarose gel electrophoresis. The PCR fragments were ethanol precipitated and resuspended in 10 µl 50% DMSO and 8 µl of the fragments was transferred to 384 well plates for microarray slide printing. The PCR fragments were arrayed onto CMT-GAPS coated microarray slides (Corning) using a Virtek ChipWriter Pro (Virtek Biotech) arrayer. Post-printing slide processing was performed by baking the slides at 80 °C for 3 hours as described in the manufacturer's technical manual.

Micoarray analysis

Cotton ovules used for RNA isolation were kept in RNAlater solution (Ambion) at 4°C overnight and then stored at -20°C. For separating the ovule outer integument from the inner tissues, ovules stored in RNAlater were used and the separation was performed under a microscope at room temperature. Total RNA isolations were performed using a method described by Wu et al., (2002). Purification of poly A^+ mRNA from total RNA was carried out using Oligotex mRNA kit (Qiagen, Cat. No. 70042), following the manufacturer's protocol. The cotton ovule cDNA microarray comprises a total of 10410 cDNA clones. Except for 52 clones encoding known cotton genes, the clones were randomly picked from cDNA libraries constructed from DP-16 ovules of –3 dpa to 0 dpa.

For microarray probe labelling, equal amounts of mRNA (0.5-1 µg) of two compared samples were reverse transcribed using Superscript II reverse transcriptase (Life Technologies), using a combination of 1 µg oligo(dT)12-18 primer and 6 µg random primers (Life Technologies) per reaction. The purification and Cy3-dUTP and Cy5-dUTP (Amersham Pharmacia Biotech) labelling of the first strand cDNA was essentially as described by Schenk et al., (2000). The labelled probes were combined and purified using a Qiaquick PCR purification kit. The conditions for slide hybridisation and washing were as described in the manufacturer's instruction manual (Corning, CMT-GAPS coated slides). The microarray images were scanned using a GenePix 4000A microarray scanner (Axon Instruments, Union CA, USA). A typical microarray comparison consisted of 4 replicates unless otherwise specified. This included two biological replicates and each biological replication contained two dye-swapped hybridisations. In a time course comparison where RNA from DP16 ovules of -4 dpa, -2 dpa and +2 dpa were compared to RNA from 0 dpa DP16 ovules, most comparisons consisted of 4 replications as mentioned above, however, self-comparison of 0 dpa to 0 dpa comprised 3 biological replications.

Scanned microarray images were analysed using the GenePix Pro program (Axon Instruments, Union CA, USA). Grids were predefined and manually adjusted to ensure optimal spot recognition and bad spots, eg. dust contamination etc., were flagged. Spots were quantified using the GenePix's fixed circle method, and medians of the fluorescence intensity of the red and green channels were used to calculate the ratio of the two channels. The data were $\log_2$ transformed and normalised using a spatial normalization method described by Wilson et al., (2003). The data were then rescaled by dividing by an estimate of the median absolute deviation (Wilson et al., 2003) before running the "find differentially expressed gene" function of tRMA (tools for R Microarray Analysis available via http://www.pi.csiro.au/gena/). For a typical microarray comparison that consisted of 4 replications, the "find differentially expressed gene" function of tRMA was used to select differentially expressed genes from each of the replications separately, and the gene lists were then compared and genes occurring in at least 3 of 4 replications were classified as differentially expressed genes for this comparison.

Gene expression in 0 dpa whole ovules of each of the mutants was compared to the wild-type DP16 at the same stage using microarray analysis as described above to identify differentially expressed genes. The number of genes identified are shown in Table 2. Each experiment was replicated a number of times as both biological replicates and dye swap replicates. The total number of cDNA clones that are differentially expressed amongst the mutants compared to the wild-type varied significantly, from an average of 60 clones in the Lintless 4A/DP16 comparison up to an average of 243 clones in the Lintless 53/DP16 comparison. In addition, the proportion of cDNA clones that are up or down-regulated also varied amongst the mutants with 4 mutant lines (1A, 53, 5B and SL) showing a higher number of cDNA clones that were up-regulated than down-regulated, and *vise versa* for the mutant Lintless 4A. These results may reflect the diverse genetic backgrounds or different genetic lesions of the mutants in addition to the variable amounts of lint produced by each line.

<u>TABLE 2.</u> Number of cDNA clones that are up- or down-regulated in each of the mutants as compared to the wild type

Comparison	Genes Differentially Expressed	Genes Up in Mutant	Genes Down in Mutant	Number of Replicates*	Minimal Reproducibility**
4A/DP16	60	9 (15%)	51 (85%)	8	75%
1A/DP16	67	48 (72%)	19 (28%)	8	75%
SL/DP16	102	88 (86%)	14 (14%)	6	83%
5B/DP16	144	91 (63%)	53 (37%)	4	75%
53/DP16	243	199 (82%)	44 (18)	4	75%

* Replicates consist both biological and technical replications. Each biological replicate (a RNA isolation) comprises two dye-swaped technical replicates. Number of biological replicates = Total number of replicates/2.
** Genes identified as being significantly differentially expressed in at least 6 out of 8 or 3 out of 4 replicates (75%), or 5 out of 6 replicates (83%).

Example 3 - Identification of genes that are differentially expressed in seed coat outer integument of lintless mutants.

Since the collected embryos described above may already have been pollinated and zygote development initiated, a separate microarray comparison was made between the mRNAs of the outer integument and those of the inner ovule tissues of the wild-type cotton, to filter out those genes that were not expressed specifically in the seed coat outer integument where fibres are initiated. Cotton ovules at 0 dpa are rapidly developing complex organs, composed of at least three separable layers of tissues: the outer integument, the inner integument and the nucellus (including a developing zygote). The genes identified as being differentially expressed in the mutant/wild-type comparisons might be constitutively expressed throughout the whole ovule or they may be expressed in only one or two of the layers. As cotton fibres develop only from the epidermal cells of the outer integument, genes that showed a higher expression level in this layer should be more relevant to fibre initiation and development compared to the genes that are predominantly expressed in the inner integument and nucellus. Outer integuments were therefore separated from the inner integuments and nucellus of 0 dpa wild type ovules by microdissection and labeled cDNA prepared from the partitioned tissues as described above. The gene expression in the outer integument was then compared to that of the inner integument and nucellus by probing the ovule cDNA microarray. The results, averaged over four replicates (two biological replicates each consisting of two dye-swapped technical replicates) revealed a total of 120 cDNA clones that were differentially expressed with 65 clones up-regulated and 55 clones down-regulated in the outer integument of wild type ovules. The list of 65 outer integument up-regulated clones was then used as a filter on the differentially expressed gene lists identified from the lintless mutant/wild type comparisons to select for cDNA clones that were up-regulated in the outer integument. This filtering resulted in the identification of a surprisingly small number of genes: 4, 7, 6, 10, 4 genes from the 1A, 4A, 5B, 53 and SL mutants respectively that were both differentially expressed in mutant/wild type comparisons and up-regulated in the outer integument.

There was significant overlap of the cDNA clones amongst the different mutants and in total only 11 unique cDNA clones were identified from this experiment as

potential candidate genes involved in early stage fibre development. The changes in relative expression in each mutant are summarized in Table 3.

TABLE 3. Genes that were up- or down-regulated in the lintless mutants and up-regulated in the outer integument[a]

Name	Clone No	SL/ DP	1A/ DP	53/ DP	4A/ DP	5B/ DP	OI/I I[c]	Most Homologous gene[d]
GhMyb25	ON035F4	-5.8	-10.1	-10.8	-10.8	-10.8	4.6	(AF336283) GHMYB25
GhFaEl	ON035N9	-5.6	-6.7	-5.7	-7	-5.8	4.7	(NP_195909) Transferase
GhFU1	ON035C9	-8.5	-11	-14.1	-15.2	-13.8	2.7	Unknown
α-Expansin	Pfs14x	-6.8	-7.6	-9.1	/	-9.1	3.3	(AF512539) Alpha-expansin
GhFU2	ON003F1	/	/	-5.9	-5.6	-8.2	2.8	Unknown
GhHD1	ON033M7	/	/	/	-5.5	-4.9	2.8	(T05850) Homeobox protein ATML1
GhTMTP	CHX015 K18	/	/	/	-8.1	-4.4	4.7	(NP_175557) ATP-dependent transmembrane transporter
GhCycD3;1	OCF07F4	/	/	/	-5.3	-3.7	4.9	(AAQ19972) Cyclin D3
CHX007 D10[b]	CHX007 D10	/	/	3.2	/	/	4.4	(AC084282) Putative protein phosphotase/(BAB 83948) CIG1
GhSus	CHX002 C10	/	/	/	/	-3.5	4.6	(AAD28641) Sucrose synthase
GhLTP	ON033M19	/	/	/	/	-5.9	3.2	(AAM62634) Lipid transfer protein

[a] The values presented in the table are the medians of Log_2 transformed, normalized and rescaled ratios of the two compared samples. The rescaling was performed by dividing through by an estimate of the median absolute deviation (Wilson et al., 2003).

[b] Most probably a chimeric clone.

[c] OI: Outer Integument; II: Inner Integument and Nucellus.

[d] Most homologous gene based on the top BlastX identity score, with Genbank Accession No. and putative biochemical function.

Except for clone CHX007D10, which appears to be a chimeric clone, that was up-regulated in mutant Lintless 53, the rest of the clones were all down-regulated in the mutants. Three genes (corresponding to clones ON035F4, ON035N9, ON035C9) were down-regulated in all 5 mutants and the other clones are down-regulated in up to 4 of the mutants. Sequence analysis showed a range of genes that had not been identified or characterized previously as important in early fibre development, including two transcription factors (GhMyb25 and a gene encoding a putative homeodomain protein); a cyclin D3 homolog; a transferase protein; a transmembrane transporter and two genes of unknown biochemical function. For ease of referring to the different genes, we have assigned them gene names that refers to their presumed functions, such as GhHD1 to refer to the cotton homeodomain protein like gene represented by ON035N9 (Table 3).

Example 4 - Expression patterns of genes in wild-type developing ovules.

RNA from DP16 ovules of -4 dpa, -2 dpa and +2 dpa was compared to RNA from 0 dpa DP16 ovules using microarrays to profile the temporal changes in expression of genes around the time of fibre initiation (DP16 time course). RNA from –2 dpa and +2 dpa ovules from the Lintless 4A mutant, which shows more severe lintless phenotypes amongst the B1278 mutants, was compared with RNA from DP ovules of corresponding stages to reveal the temporal profiles of the genes inhibited in mutant 4A (4A/DP multi-time point comparison). The results of these experiments for the identified candidate genes are shown in Fig. 1.

The DP16 time course showed two classes of expression profiles: Class I genes showed peak expression at 0 dpa; while Class II genes exhibited increased expression towards +2 dpa (Fig. 1. Column A). Three genes, GhMyb25, the GhHD1 and GhCycD3;1, had a Class I expression profile suggesting a role in the early events of fibre initiation at anthesis. The expression of GhCycD3;1 increased continually from –4 dpa to 0 dpa and plateaued between 0 dpa and +2 dpa, while GhMyb25 and the GhHD1 exhibit a dip in expression at –2 dpa followed by a peak at 0 dpa and then a decline towards +2 dpa. The peak expression at 0 dpa of these three genes coincided with the time of fibre initiation. The rest of the genes all showed a Class II expression pattern although the specific details differed among them. The expression of GhFU1 and GhSus (sucrose synthase) increased gradually in the time period examined. GhFaEl (transferase family) and the expression of GhTMTP (transmembrane transporter)

showed a slight decrease from –4 dpa to –2 dpa and then increased gradually towards +2 dpa. The third group from this class comprised α-Expansin and GhLTP (lipid transfer protein) which show a distinctly flat profile from –4 dpa to 0 dpa followed by a sharp increase from 0 dpa to +2 dpa. The last member from this class, GhFU2, exhibited increased expression from –4 dpa to –2 dpa and again from 0 dpa to +2 dpa, while the expression between –2 dpa to 0 dpa remained unchanged.

The multiple time point comparisons of 4A/DP16 revealed the time and duration of up- or down-regulation of the genes in the 4A mutant relative to DP16 (Fig. 1. Column B). Among the Class I genes, none showed significant repression relative to DP16 at –2 dpa in 4A ovules, instead, the repression started after –2 dpa and reached the lowest level at 0 dpa for the GhHD1 and GhCycD3;1 genes, while GhMyb25 continued to decrease slightly after 0 dpa. Class II genes, in a similar fashion, did not show any significant repression at –2 dpa, and repression started after –2 dpa for most of the genes except GhSus, which only showed a later repression after 0 dpa. All the Class II genes exhibited repression at about +2 dpa. Three Class II genes, GhSus, α-Expansin and GhLTP, were not identified as differentially expressed genes in the initial 0 dpa 4A/DP comparisons. It became clear from this experiment that the repression of GhSus did not occur until after 0 dpa and only became significant at +2 dpa, while the repression of α-Expansin and GhLTP began after –2 dpa only became highly significant towards +2 dpa when fibres were rapidly elongating in the wild-type.

Example 5 - Confirmation of down-regulation of fibre initiation genes.

The genetic background of the fibre mutants (B1278 and SL) used herein was not identical to that of the DP16 control used in the comparisons. Neither parental genotype was available for use in the experiments described above. While the differential expression of genes observed between the mutants and the wild-type might have arisen due to differences in the genetic backgrounds of the plants, the commonality of the genes identified among the different mutants and the fact that some have been characterized previously as important for fibre development using different strategies, suggested otherwise. A *fuzzless-lintless* (*fl*) mutant had recently been isolated from the Chinese *G. hirsutum* cultivar Xu-142 and used to identify and characterize fibre development related genes (Yu et al., 2000, Li et al., 2002, Ji et al., 2003). These two lines provided an isogenic pair with which to validate the genes identified from the other mutants. RNA from 0 dpa ovules of *fl* was compared to that of 0 dpa ovules of Xu-142 and 119 clones were identified as differentially expressed in four replicates. The same outer/inner integument gene expression filter as described above was applied to the data set and identified 13 differentially expressed genes that were also up-regulated in the outer integument of DP16 ovules. Amongst the 13 genes, 8 were in common with genes

identified in the other lintless mutant/DP16 comparisons and the results are presented in Table 4. This comparison also revealed 5 additional genes that had not been identified in the previous 5 mutants. One of the cDNA clones, ON038N8 (886 bp), encodes a Myb protein which was 69% identical to the GhMyb25 protein. Other genes include two different RD22 genes, a second LTP and a putative L-asparaginase.

TABLE 4. Genes differentially expressed in *fl* as compared to Xu-142

Name	Clone No	fl/Xu-142 **	Most Homologous gene***
α-Expansin*	Pfs14x	-11.5	(AF512539) Alpha-expansin
GhFU1*	ON035C9	-9.8	Unknown
GhMyb25*	ON035F4	-9.0	(AF336283) GHMYB25
GhFU2*	ON003F1	-7.8	Unknown
GhFaEl*	ON035N9	-7.4	(NP_195909) Transferase
GhHD1*	ON033M7	-6.1	(T05850) Homeobox protein ATML1
GhLTP*	ON033M19	-4.9	(AAM62634) Lipid transfer protein
GhTMTP*	CHX015K18	-3.7	(NP_175557) ATP-dependent transmembrane transporter
GhMyb25-like	ON038N8	-4.4	(AF336283) GHMYB25
GhRD22	OCF005C10	-4.4	(AAL67991). Dehydration-induced protein RD22
GhAsp	OCF008G9	-3.4	(BAC66615) L-aspraginase
GhLTP2	OCF010D8	3.4	(CAA65477) Non-specific lipid-transfer protein
GhRD22-like	OCF006C1	5.3	(BAC22498) Resistant specific protein-1

* Genes in common with the candidate genes from the other 5 mutants.

** The values presented in the table are the medians of Log_2 transformed, normalized and rescaled ratios of the two compared samples. The rescaling was performed by dividing through by an estimate of the median absolute deviation (computed on the final residual mean-difference data) as described by Wilson et al., (2003).

*** Based on the top BlastX hit.

Example 6 - Characterisation of GhMyb25 expression.

The nucleotide sequence of the clone ON035F4 was obtained. It was 1160 nucleotides in length with a coding region from nucleotides 68 to 995, encoding a protein which was 98% identical at the amino acid level to GhMyb25 (AF336283), expressed in 0 dpa ovules of *G. hirsutum* cultivar Acala Maxxa (Benjamin Burr, in

Genbank). The encoded protein was an R2R3 type of Myb transcription factor. It was also 96% identical to the *G. arboretum* EST (BE054276), suggesting that it was from the A-genome present in tetraploid cotton. Outside the R2R3 region, which is highly conserved amongst all Myb transcription factors, GhMyb25 showed highest homology to the *Petunia hybrida* MYB.Ph3 and *Antirrhinum majus* MIXTA (AmMIXTA) than to Arabidopsis GL1 and cotton MYBA, another cotton myb which caused distinct abnormalities when over expressed in transgenic tobacco including the production of cotyledonary trichomes (Payne et al., 1999). The sequence of GhMyb25 currently in Genbank (AF336283) contained an unspliced intron that was not present in our clone (nucleotides 201-282 of AF336283).

Reverse transcription-polymerase chain reaction (RT-PCR) assays were used to analyse expression of the gene as follows. Total RNA samples isolated from cotton tissues were DNase (RQ1 RNase-free DNase, Promega) treated and 0.5 μg of the total RNA was used in a RT-PCR reaction. The first strand cDNA synthesis was performed using SuperScript II reverse transcriptase and buffer supplied by the manufacturer (Life Technologies). The RT-PCR reaction was performed essentially as described by McFadden et al., (2001). The cotton β-tubulin gene was used as a control in all the RT-PCR reactions. The forward and reverse primers used for β-tubulin are 5'-AGAACATGATGTGTGCTGC-3' (SEQ ID NO:65) and 5'-AGCTGTGAACTGCTCACTC-3' (SEQ ID NO:66) respectively and the resulting cDNA fragment was 300 bp. The forward and reverse primers used for GhMyb25 RT-PCR were: 5'-TCAAACCCTCCTCAAAGCAACC-3' (SEQ ID NO:67) and 5'-ATTCCATTACCAGACGATGATGAC-3' (SEQ ID NO:68) respectively and this produced a cDNA fragment of 224bp. The GhMyb25 and β-tubulin RT-PCR reactions were performed in a one-tube reaction amplified with an initial denaturation cycle at 95°C for 3 min followed by 23 cycles at 95°C for 15 sec, 55 °C for 15 sec, 72 °C for 1 min and with a final cycle of 72°C for 2 min. 5 μl of the RT-PCR reaction was checked on a 2% agarose gel and the gel was Southern-blotted to Hybond-N$^+$ membrane (Amersham Pharmacia Biotech) and hybridized with ^{32}P- labelled probe derived from the cDNA clone of GhMyb25.

GhMyb25 expression was detected in 0 dpa wild-type ovules and not in petal, leaf and stem using RT-PCR (Fig. 2, panel a). GhMyb25 expression was detected in –2, 0, 2 and 5 dpa ovules with highest expression in 0 and 2 dpa ovules (Fig. 2, panel b). No expression of GhMyb25 was visible in ovules of –2, 0 and 2 dpa from three of the lintless mutants, 1A, SL1-7-1 and 4A (Fig. 2, panel c) although very low expression in the mutant ovules was revealed after hybridizing RT-PCR products with an ON035F4 probe (Fig. 2, panel d), consistent with the low levels of lint production that still occurs

on these ovules. GhMyb25 expression peaked at 0 dpa in lines 1A and SL1-7-1, whereas in 4A, the highest expression was detected slightly later at 2 dpa.

Example 7 - Characterisation of the homeodomain protein gene GhHD1.

ON033M7, a partial cDNA clone of 442 nucleotides, contained a small region at the 3'end of its encoded protein with homology to two homeodomain proteins including protodermal factor 2 (NP_567274) (26/28 identical amino acids) and the L1-specific and ovule specific homeodomain gene ATML1 (T05850) (26/30, 86%). ATML1 has been grouped with the *Arabidopsis* GLABRA2 in the same HD-GL2 class and they also share a common L1 layer-specific or dermal-specific pattern of expression (Lu et al., 1996). The cotton gene was designated GhHD1.

RT-PCR experiments were carried out to analyse expression, in similar fashion to those described above. The forward and reverse primers used for the GhHD1 RT-PCR were: 5'-GCTTTCTCTTGGATCAG-3' (SEQ ID NO:69) and 5'-CAATAACACATGAAACCAG-3' (SEQ ID NO:70) respectively and these resulted in a cDNA fragment of 384bp. The GhHD1 and β-tubulin RT-PCR reactions were performed separately under the conditions described above. 10 μl of the RT-PCR reaction was electrophoresed on a 2% agarose gel and the gel was Southern-blotted to Hybond-N^+ membrane (Amersham Pharmacia Biotech) and hybridized with ^{32}P-labelled probe derived from the cDNA clone of the putative homeodomain gene. Since the expression of the β-tubulin appeared to be variable in different cotton tissues, the quantification of the GhHD1 expression using β-tubulin gene as a standard was only performed on the 0 dpa ovules of different cotton lines using an Image-Quant program (Molecular Dynamics).

GhHD1 was mainly expressed in ovules of various developmental stages and at much lower levels in leaves as revealed by RT-PCR (Fig. 3). The expression increased at –1 dpa and remained high till 2 dpa. Low expression levels were observed in 4 dpa ovules (with fibres attached), 8 dpa ovules (without fibres) and 8 dpa detached fibres. The expression levels of 0 dpa ovules of mutants 5B and 4A relative to DP16 0 dpa ovules after normalization with β-tubulin expression is shown in Fig. 3. The expression in the mutant ovules was slightly reduced in 5B and remained similar to wild-type in 4A ovules. The RT-PCR band was confirmed as GhHD1-specific by Southern blot hybridization using the ON033M7 cDNA fragment as probe.

Example 8 - Characterisation of other genes.

The characteristics of the genes identified in this study are summarized in Tables 5 and 6.

TABLE 5. Characteristics of fibre initiation genes identified from cotton ovules.

Designation	Clone No	Length (nt*)	Full Length Or Partial?	Translation start/stop	Translation product size
GhMyb25	ON035F4	1160	F	68/995	309
GhFaEl	ON035N9	704	P	-	at least 234
GhFU1	ON035C9	548	F	50/371	107
α-Expansin	Pfs14x[b]	835	P	?/766	255
GhFU2	ON003F1	727	P	?/563	113
GhHD1	ON033M7	2222	P	?/1873	624
GhTMTP	CHX015K18	985	P	?/572	191
GhCycD3;1	OCF07F4	600	P	?/453	151
CHX007D10[c]	CHX007D10		Chimerical ?	-	
GhSus	CHX002C10	2611	F	8/2423	805
GhLTP	ON033M19	732	F	10/595	195
GhMyb25-like	ON038N8	886	P	?/887	295
GhRD22	OCF005C10	1353	F	58/1180	374
GhAsp	OCF008G9	1024	P	?/796	265
GhLTP2	OCF010D8	610	F	33/402	123
GhRD22-like	OCF006C1	1373	P	?/1213	404

* Omitting polyA sequence if present.

TABLE 6. Summary of closest match for the cotton ovule genes.

Name	Clone No	Most Homologous gene (BlastX)[a]	Percentage Identity by BlastX (Similarity)[a]	Accession No. of nearest match (BlastN)[b]	Percentage identity (No. of matched nucleotides)	Percentage identity over full length
GhMyb25	ON035F4	(AF336283) GHMYB25 [Gossypium hirsutum]	98% (98%)	AF336283 G. hirsutum GHMYB	99% (951/964)	99%
GhFaEl	ON035N9	(NP_195909) Transferase family [Arabidopsis thaliana]	54% (72%)	NM_120367.2| A. thaliana transferase	81% (88/108)	58%
GhFU1	ON035C9	none	none	AF027686 Onobrychis viciifolia	81% (89/109)	50%
α-Expansin	Pfs14x	(AF512539) Alpha-expansin precursor [Gossypium hirsutum]	99%	AF043284 G. hirsutum expansin	99% (828/836)	99%
GhFU2	ON003F1	none	none	none	none	-
GhHD1	ON033M7	(T05850) Homeobox protein ATML1 [Arabidopsis thaliana]	86% (93%)	NM_116727.2| ; A. thaliana homeodomain	79% (482/605)	70%
GhTMTP	CHX015K18	(NP_175557) ATP-dependent transmembrane transporter [Arabidopsis thaliana]	65% (86%)	NM_104024.2|	87% (68/78)	63%
GhCycD3;1	OCF07F4	(AAQ19972) Cyclin D3-2 [Euphorbia esula]	53% (71%)	NM_119579.2|	91% (31/34)	52%
CHX007D10	CHX007D10	(BAB83948) proline oxidase/dehydrogenase		AY492003.1| G. max proline dehydrogenase	80% (120/149)	40%
GhSus	CHX002C10	(AAD28641) Sucrose synthase [Gossypium hirsutum]	94% (95%)	U73588 G. hirsutum sucrose synthase	98% (2443/2482)	98%
GhLTP	ON033M19	(AAM62634) Lipid transfer protein,	66%	none	none	

		putative [Arabidopsis thaliana]	(83%)			
GhMyb25-like	ON038N8	(AF336283) GHMYB25 [Gossypium hirsutum]	58% (64%)	AF336283 G. hirsutum GHMYB	94% (64/68)	63%
GhRD22	OCF005C10	(AAL67991) Dehydration-induced protein RD22 [Gossypium hirsutum]	76% (80%)	AY072821.1| G. hirsutum dehydration induced	94% (810/864)	94%
GhAsp	OCF008G9	(BAC66615) L-aspraginase [Glycine max]	63% (76%)	AP006428.1| Lotus corniculatus Chromosome5 complete sequense 127049bp	93% (56/60)	
GhLTP2	OCF010D8	(CAA65477) Non-specific lipid-transfer protein [Prunus dulcis]	54% (74%)	AF519812.1| Nicotiana tabacum	91% (41/45)	54%
GhRD22-like	OCF006C1	(BAC22498) Resistant specific protein-1 [Vigna radiata]	43% (60%)	none	30/32, coincidental	

[a] BlastX determines the percentage amino acid identity (%similarity in parentheses) over the region of closest match to the Genbank database.

[b] BlastN determines the nearest match at the nucleotide level in the Genbank database.

Clone OCF07F4 encoded a cyclin D3, similar to AAQ19972, 106/149 similar amino acids, 71%).

Example 9 - Fibre cells undergo DNA endoreduplication during initiation.

The observation that a cyclin D3 gene encoded by clone OCF007F4 was down-regulated in 0 dpa ovules of mutants 4A and 5B compared to DP16 prompted the inventors to investigate the cell division and DNA replication activities of the epidermal layers of DP16 and lintless 4A ovules. Ovules of cotton line DP16 and 4A at -2, -1,and 0 dpa stages were fixed in 3:1 (95% ethanol:acetic acid) for 1hr at room temperature, cleared in 95%/1mM $MgCl_2$ ethanol over night at room temperature and rehydrated through an ethanol series to 10mMTris/1mM $MgCl_2$ according to Szymanski and Marks (1998). The ovules were stained in 0.1µg/ml propidium iodide for 30 seconds and than distained and kept in 10mMTris/1mM $MgCl_2$.

Nuclear DNA content of ovule epidermal and fibre cells at the chalazal end were measured using a Leica SP2 confocal laser scanning microscope (Leica, Wetzler, Germany). At least 200 nuclei were measured from each sample which consisted of at least 3 ovules. Fluorescence at 600-740 nm was collected after excitation at 488 and 543 nm using a 63 X NA 1.25 water-immersion lens. After optically sectioning through the ovule epidermis, the mean fluorescence intensity and dimensions of epidermal and fibre cell nuclei was measured from the maximum projection of the optical stack. Total fluorescence of individual nuclei was calculated by multiplying nuclear area by average fluorescence. This value was converted to a ratio by normalising against total fluorescence of epidermal cell nuclei at telophase or anaphase (2C) within the same image. The normalized values were then used to construct histograms of epidermal and fibre cell nuclear DNA content.

Since fibre cells are known to cease dividing after differentiation, the ovule epidermal cell division activities of DP16 and 4A-183 were examined at and before anthesis. Over the period examined (-2, -1 and 0 dpa), the extent of cell division in the ovule epidermis of lintless 4A and DP16 were not significantly different (paired t-test, P = 2.1%) although the division rate in 4A was slightly higher than in DP16 (Table 7). It appears that the ovule epidermis division rates are higher at –1 dpa for both DP16 and 4A, although the significance of this is unclear.

TABLE 7. Cell division rates in ovule epidermal cells of DP16 and mutant 4A

DPA	DP16	4A
-2 dpa	2.78%	2.97%
-1 dpa	3.25%	3.5%
0 dpa	2.69%	2.84%

Relative DNA contents of the epidermal cells and fibre cells were also measured, and normalized using DNA contents of nuclei at anaphase or telophase (2C) and the results are presented in Fig. 4. Since the pre-fibre initials and epidermal cell are visually indistinguishable at –2 and –1 dpa, the data for those times are presented as total epidermal cells. The results indicate that epidermal pavement cells of DP16 and Lintless 4A at these time points have a DNA content peak around 2 to 2.4C, while differentiated fibre initials of DP16 when clearly distinguishable at 0 dpa, have an increased DNA content with the majority of cells showing a DNA content between 2.8 C and 5.2C. While this result clearly suggests that the majority of fibre cells undergo at least one round of DNA endoreduplication during initiation, the involvement of the cyclin D3 gene in this process still needs to be verified.

Example 10 - Cloning of full-length cDNA sequences and genes encoding therefor

At least two approaches can be used to determine the full length sequence of partial cDNA clones described herein.

One method is to screen a cDNA library, such as the cotton DP-16 ovule -3 dpa to 0 dpa library described herein, with a radioactively labelled polynucleotide which comprises the known portion of the cDNA. Library screening is performed as described by Sambrook et al., (supra), or other techniques known to those of skill in the art.

In another method, two primers of about 17 to about 20 nucleotides derived from both ends of the known partial sequence are synthesized and used to amplify the desired cDNA from a population of cDNA reverse transcribed with a poly-T comprising primer from mRNA obtained from, for example, cotton DP-16 ovule -3 dpa to 0 dpa. The polymerase chain reaction (PCR) is carried out under routine conditions, for instance, in 25 µl of reaction mixture with 0.5 µg of the above cDNA mixture. A convenient reaction mixture is 1.5-5 mM $MgCl_2$, 0.01% (w/v) gelatin, 20 µM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated

thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight (as determined by Northen blot analsysi) is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several other methods are available for the identification of the 5' or 3' ends of an mRNA sequence. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length cDNA.

To clone the corresponding gene of a cDNA described herein, a cotton genomic library is made in a λ vector and plaques obtained by plating 10^6 or more λ infectious particles at high density in a suitable *E. coli* host. The plaques are transferred to nylon filters. A gene specific probe designed considering the polynucleotides provided herein is labelled with radioactive label and used to hybridise to the nylon filters. Plaques corresponding to spots of hybridisation are isolated and confirmed to be positive for desired sequence by second or third rounds of hybridisation. DNA sequencing of the gene segments in the λ clones is carried out by standard methods to determine the full nucleotide sequence of the gene and the flanking upstream and downstream regions.

Example 11 - Production of transgenic cotton

The coding sequence of a gene of the invention is operably linked to a subterranean clover stunt virus promoter (S7; WO 96/06932) and a 3' transcription termination and polyadenylation signal functional in plants. This chimeric gene is operably linked to a selectable marker gene and introduced into a T-DNA vector. Cotton plants are transformed using the Agrobacterium mediated transformation technique. Transgenic cotton lines are identified, fibre number, fibre length, fuzz fibre length, cellulose content, and dry weight of the lint is analyzed.

Example 12 - Discussion

The use of multiple mutant lines in the above mentioned expression studies enabled confirmation and complementation of the findings from one mutant to another and focus in on the most critical genes for fibre development. It also helps to smooth out "noise" contributed by the biological variability in fibre growth and the unknown and maybe diverse genetic backgrounds of some of the mutants. The comparison of gene expression profiles between the outer integument and the inner ovule tissues served as a filter, to eliminate those genes that are not expressed at higher levels in the outer integument and helped to focus on a small set of about 10 candidate genes. The wild type time course data reveal that the expression profiles of these candidate genes separated them into two classes with class I genes (GhMyb25, GhHD1 and GhCycD3;1) showing peak expression at 0 dpa, coinciding with the time of fibre initiation; class II genes exhibiting increased expression at 2 dpa, suggesting a more important role in fibre elongation.

By analogy with the regulatory genes involved in Arabidopsis leaf trichome development, it might have been expected to find a Myb transcription factor expressed in cotton fibre that was similar to GL1. GhMyb25 (and the GhMyb25-like gene), however, shows higher sequence similarity to the *Petunia hybrida* Myb.Ph3 and the *Antirrhinum* MIXTA than to GL1 or other cotton Mybs. Both Myb.Ph3 and MIXTA show petal epidermis-specific expression. Based on its expression pattern, it was speculated that the function of Myb.Ph3 was to regulate flavonoid biosynthesis (Solano et al., 1995), but this speculation has not been confirmed. The function of MIXTA has been revealed as a controlling factor for the conical shape of petal epidermal cells (Noda et al., 1994). Over-expression of MIXTA in transgenic tobacco lead to production of supernumerary trichomes on cotyledons, leaves and stems as well as novel production of conical cells on leaves (Payne et al., 1999). In contrast, GhMyb25 is expressed only in the ovules and not in later stage fibres (or petals so is not a homolog of MIXTA). It had higher expression in outer integuments and the time of expression coincide with fibre initiation. Accordingly, GhMyb25 plays a role in fibre initiation. The fact that GhMyb25 is down-regulated in all the lintless mutants, including the new *fl* mutant, points to a role as a positive regulator of fibre initiation.

Comparison of the *fl* mutant with its parental genotype identified in addition to the 8 genes common to other mutants, a second Myb transcription factor, a GhMyb25-like gene, containing a conserved region outside the R2R3 domain shared by all the MIXTA class of Mybs (Stracke et al., 2001). The GhMyb25-like gene is only 64% identical to probable A-genome derived GhMyb25 at the nucleotide level (69%

similarity at the amino acid level), suggesting it is unlikely to be the homoelogous D-genome partner of GhMyb25 present in tetraploid cotton. GhMyb25-like is expressed at low level in -4, -2 dpa ovules of DP16 (0.3 relative to 0 dpa ovule of 1) and increased sharply to 1 at 0 dpa and remained at a similar level of 1 at 2 dpa. This expression profile indicates a role for GhMyb25-like in fibre initiation. Among the 5 additional genes identified in this analysis, there were two RD22 genes, consistent with the findings of Li et al., (2002) who identified a RD22 gene showing fibre specific expression using the same lines.

The putative homeodomain gene identified in this study has high similarity in part of its C-terminus to the L1 specific and ovule specific homeodomain gene ATML1. ATML1 was classified in the same HD-GL2 class as Arabidopsis GL2 based on sequence homology and they share a common L1 layer-specific or dermal-specific pattern of expression (Lu et al., 1996). ATML1 was proposed to be involved in setting up morphogenetic boundaries of positional information necessary for controlling cell specification and pattern formation based on gene expression patterns. GL2 that has been studied for its role in trichome, root-hair and seed coat development (Rerie et al., 1994, Cristina et al., 1996, Masucci et al., 1996). The GL2 mutations resulted in aborted trichomes with aberrant cell expansion whereas entopic expression noticeably increased the number of trichomes and induced clusters of trichome formation (Ohashi et al., 2002). The GhHD1 gene is expressed in ovules with higher expression in outer integument, in fibres as well as in leaves and this expression pattern probably reflects a more general role in different epidermal cell specification and pattern formation similar to that shown by the GL2.

DNA endoreduplication, a strategy to amplify nuclear DNA without cell division is a major mechanism leading to somatic polyploidisation in plants (reviewed by Joubès and Chevalier 2000). Correlations have been established between polyploidy and cell differentiation and cell expansion. While it is well established that Arabidopsis trichomes undergo four rounds of endoreduplication during development, leading to branched cells with nuclei containing about 32C DNA (Schnittger et al., 2002), it has been less than clear whether cotton fibre initials undergo a similar process. Berlin (1986) studied tritiated thymidine uptake by epidermal layer using *in vitro* cultured cotton ovules and observed that there was an increase in thymidine incorporation from –2 dpa to 1 dpa and then the incorporation declined and finally stopped at 6 dpa. These observations were interpreted as DNA synthesis in preparation for cell division. Since fibre initials do not undergo divisions and no thymidine incorporation was observed in the elongating fibres, the author suggested that gene

amplification did not occur during fibre development over the time observed (Berlin 1986). Van't Hof (1998) reported that the DNA content of developing cotton fibre cells only increased by about 24% from 2 dpa to 5 dpa and suggested that during early stages of development fibre cell nuclei either selectively amplify certain sequences or enter S-phase replicating only a portion of their genome. Using laser-confocal microscopy and propidium iodide staining, we examined ovule epidermal cell division rates and DNA contents of epidermal cells and fibre cells. Our results show that the epidermal cell division rates remain relatively constant from –2 dpa to 0 dpa (with a small increase at –1 dpa). While nuclear DNA contents of epidermal cells remain largely unchanged from –2 dpa to 0 dpa, the fibre initials contain nuclei that mostly show higher than 2C DNA content with the majority of cells showing DNA contents between 2.8 C and 5.2C. While our results suggest the fibre initials undergo one round DNA endoreduplication starting at 0 dpa, they do not exclude the possibility of selective amplification of certain sequences or partial replication of the genome at later stages suggested by Van't Hof's work. In addition, the enlarged nuclei in fibre initials revealed by the ultrastructural studies (review by Berlin 1986) provided further support to the DNA amplification phenomenon.

Although accumulating data reveal that DNA endoreduplication is developmentally regulated, it is still poorly understood in plants (reviewed by Joubès and Chevalier 2000). Assuming the endoreduplication is a modified cell cycle, it may share common determinants with the classic cell cycle (Joubès and Chevalier 2000). The two main control points in the cell cycle are at the G1/S and G2/M transitions and in most plant cell types, the primary control point probably operates during G1 phase. This period not only includes the point of commitment to cell division, but may also represent the time during which differentiation decisions are made (reviewed by Meijer and Murray 2000). Mammalian cyclin D-Cdk4 complexes have been characterized as growth factor-responsive cell cycle regulators operating during G1 phase. Cyclin D3 was found to be present at high levels in megakaryocytes undergoing endoreduplication and was upregulated following exposure to the proliferation, maturation and ploidy-promoting factor, Mpl ligand (Zimmet et al., 1997). In plants, the presence of mutiple Cyclin D3 genes raises the question of functional redundancy of these genes and the extent to which they may have distinct or overlapping roles (Meijer and Murray, 2000). Arabidopsis CycD3;1, which is highly cytokinin-inducible (Riou-Khamlichi et al. 1999), when ectopically expressed, induced not only DNA replication but also cell division in trichomes (Schnittger et al., 2002). In synchronized tobacco BY-2 cell suspension cultures, tobacco CycD3; 2 was induced in G1 and remained at a constant

level through out the cell cycle, similar to mammalian D-type cyclins. In contrast, CycD3;1 transcripts accumulated during mitosis, a pattern of expression not normally associated with D-type cyclins, suggesting a novel role for plant cyclins during mitosis or alternatively a BY-2 cell-specific phenomenon and not a normal feature of plant cell-cycle progression (Sorrell et al., 1999). The GhCycD3.1 identified in our experiments shows highest sequence homology to *Euphobia esula* cyclin D3;2. Since the decreased expression of this gene in the outer integuments of lintless mutant 4A-183 did not affect the epidermal cell division rates, it is appears that this gene is involved in the DNA endoreduplication of fibre initials similar to the tomato CycD3;1's involvement in endoreduplication of the differentiated giant cells of the fruit gel tissue (Joubès et al., 2000).

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

All publications discussed above are incorporated herein in their entirety.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

Dated this thirty-first day of March 2004

Commonwealth Scientific and Industrial Research Organisation

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CLAIMS

1. A method of altering fibre initiation and/or elongation in a fibre producing plant comprising manipulating said plant such that the production of a polypeptide is modified when compared to a wild-type plant, wherein the polypeptide comprises a sequence selected from the group consisting of:

i) an amino acid sequence provided as any one of SEQ ID NO's:1 to 16; or

ii) an amino acid sequence which is at least 50% identical to any one of SEQ ID NO's:1 to 16.

2. The method of claim 1, wherein the polypeptide comprises a sequence selected from the group consisting of:

i) an amino acid sequence provided as SEQ ID NO: 1; or

ii) an amino acid sequence which is at least 80% identical to SEQ ID NO:1.

3. The method of claim 1, wherein the polypeptide comprises a sequence selected from the group consisting of:

i) an amino acid sequence provided as SEQ ID NO:2; or

ii) an amino acid sequence which is at least 80% identical to SEQ ID NO:2.

4. The method of claim 1, wherein the polypeptide comprises a sequence selected from the group consisting of:

i) an amino acid sequence provided as SEQ ID NO:3; or

ii) an amino acid sequence which is at least 80% identical to SEQ ID NO:3.

5. The method according to any one of claims 1 to 4 which comprises recombinantly expressing the polypeptide in said plant.

6. The method according to any one of claims 1 to 4 which comprises reducing the level of the polypeptide endogenously produced by the plant.

7. The method of claim 6, wherein the level of the polypeptide endogenously produced by the plant is reduced by exposing the plant to an antisense polynucleotide or a catalytic polynucleotide which hybridizes to an mRNA molecule encoding the polypeptide.

8. The method of claim 6, wherein the level of the polypeptide endogenously produced by the plant is reduced by exposing the plant to a dsRNA molecule that specifically down-regulates mRNA levels in a cell of an mRNA molecule encoding the polypeptide.

9. The method according to any one of claims 1 to 8, wherein the plant is a horticultural plant.

10. The method according to any one of claims 1 to 8, wherein the plant is a species of the Genus *Gossypium*.

11. A method of assessing the potential of a fibre producing plant to produce fibre, the method comprising analysing the plant for a genetic variation in a polynucleotide associated with fibre initiation and/or elongation, wherein the polynucleotide comprises a sequence selected from the group consisting of:

i) a nucleotide sequence provided as any one of SEQ ID NO's:17 to 45; or

ii) a nucleotide sequence which is at least 50% identical to any one of SEQ ID NO's:17 to 45.

12. The method of claim 11 which comprises performing an amplification reaction on nucleic acids obtained from said plant, or nucleic acids synthesized using nucleic acids from said plant as a template, wherein the production of an amplicon in said amplification reaction indicates an association with fibre producing potential.

13. The method of claim 11 which comprises performing an amplification reaction on nucleic acids obtained from said plant, or nucleic acids synthesized using nucleic acids from said plant as a template, wherein the lack of production of an amplicon in said amplification reaction indicates an association with fibre producing potential.

14. The method of claim 11 which comprises performing a hybridization reaction on nucleic acids obtained from said plant, or nucleic acids synthesized using nucleic acids from said plant as a template, wherein a detectable signal produced by the hybridization reaction indicates reduced fibre producing potential.

15. The method of claim 11 which comprises performing a hybridization reaction on nucleic acids obtained from said plant, or nucleic acids synthesized using nucleic acids

from said plant as a template, wherein the lack of a detectable signal by the hybridization reaction indicates reduced fibre producing potential.

16. The method of claim 11, wherein the polynucleotide is mRNA and the method comprises determining the levels of mRNA of the polynucleotide in the plant ovule at, or around, anthesis.

17. A method of assessing the potential of a fibre producing plant to produce fibre, the method comprising analysing the plant for polypeptide involved in fibre initiation and/or elongation, wherein the polypeptide comprises a sequence selected from the group consisting of:

i) an amino acid sequence provided as any one of SEQ ID NO's:1 to 16; or

ii) an amino acid sequence which is at least 50% identical to any one of SEQ ID NO's:1 to 16.

18. The method of claim 17, wherein the method comprises determining the levels of the polypeptide in the plant ovule at, or around, anthesis.

19. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:1,

ii) a polypeptide comprising an amino acid sequence which is at least 87% identical to SEQ ID NO:1, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

20. The polypeptide of claim 19, wherein the polypeptide comprises an amino acid sequence which is at least 95% identical to SEQ ID NO:1.

21. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:2, and

ii) a biologically active fragment of i),

wherein the polypeptide regulates fibre initiation and/or elongation.

22. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:3,

ii) a polypeptide comprising an amino acid sequence which is at least 54% identical to SEQ ID NO:3, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

23. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:4,

ii) a polypeptide comprising an amino acid sequence which is at least 55% identical to SEQ ID NO:4, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

24. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:5,

ii) a polypeptide comprising an amino acid sequence which is at least 50% identical to SEQ ID NO:5, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

25. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:6,

ii) a polypeptide comprising an amino acid sequence which is at least 50% identical to SEQ ID NO:6, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

26. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:7,

ii) a polypeptide comprising an amino acid sequence which is at least 79% identical to SEQ ID NO:7, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

27. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:8,

ii) a polypeptide comprising an amino acid sequence which is at least 66% identical to SEQ ID NO:8, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

28. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:9,

ii) a polypeptide comprising an amino acid sequence which is at least 95% identical to SEQ ID NO:9, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

29. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:10,

ii) a polypeptide comprising an amino acid sequence which is at least 67% identical to SEQ ID NO:10, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

30. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:11,

ii) a polypeptide comprising an amino acid sequence which is at least 55% identical to SEQ ID NO:11, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

31. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:12,

ii) a polypeptide comprising an amino acid sequence which is at least 59% identical to SEQ ID NO:12, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

32. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:13,

ii) a polypeptide comprising an amino acid sequence which is at least 77% identical to SEQ ID NO:13, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

33. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:14,

ii) a polypeptide comprising an amino acid sequence which is at least 50% identical to SEQ ID NO:14, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

34. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:15,

ii) a polypeptide comprising an amino acid sequence which is at least 64% identical to SEQ ID NO:15, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

35. A substantially purified polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence as provided in SEQ ID NO:16,

ii) a polypeptide comprising an amino acid sequence which is at least 50% identical to SEQ ID NO:16, and

iii) a biologically active fragment of i) or ii),

wherein the polypeptide regulates fibre initiation and/or elongation.

36. The polypeptide according to any one of claims 19 to 35, wherein the polypeptide can be purified from a species of the Genus *Gossypium*.

37. The polypeptide according to any one of claims 19 to 36, which is a fusion protein further comprising at least one other polypeptide sequence.

38. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:17;

ii) a sequence of nucleotides as provided in SEQ ID NO:18;

iii) a sequence encoding a polypeptide according to claim 19 or claim 20;

iv) a sequence of nucleotides which is at least 87% identical to SEQ ID NO:17 or SEQ ID NO:18; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:46.

39. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:19,

ii) a sequence of nucleotides as provided in SEQ ID NO:20,

iii) a sequence encoding a polypeptide according to claim 21, and

iv) a sequence complementary to any one of i) to iii).

40. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:21;

ii) a sequence of nucleotides as provided in SEQ ID NO:22;

iii) a sequence encoding a polypeptide according to claim 22;

iv) a sequence of nucleotides which is at least 54% identical to SEQ ID NO:21 or SEQ ID NO:22; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:47.

41. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:23;

ii) a sequence encoding a polypeptide according to claim 23;

iii) a sequence of nucleotides which is at least 55% identical to SEQ ID NO:23; and

iv) a sequence which hybridizes to any one of i) to iii) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:48.

42. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:24;

ii) a sequence of nucleotides as provided in SEQ ID NO:25;

iii) a sequence encoding a polypeptide according to claim 24;

iv) a sequence of nucleotides which is at least 50% identical to SEQ ID NO:24 or SEQ ID NO:25; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:49.

43. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:26;

ii) a sequence of nucleotides as provided in SEQ ID NO:27;

iii) a sequence encoding a polypeptide according to claim 25;

iv) a sequence of nucleotides which is at least 50% identical to SEQ ID NO:26 or SEQ ID NO:27; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:50.

44. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:28,

ii) a sequence of nucleotides as provided in SEQ ID NO:29,

iii) a sequence encoding a polypeptide according to claim 26,

iv) a sequence complementary to any one of i) to iii).

45. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:30;

ii) a sequence of nucleotides as provided in SEQ ID NO:31;

iii) a sequence encoding a polypeptide according to claim 27;

iv) a sequence of nucleotides which is at least 65% identical to SEQ ID NO:30 or SEQ ID NO:31; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:51.

46. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:32,

ii) a sequence of nucleotides as provided in SEQ ID NO:33,

iii) a sequence encoding a polypeptide according to claim 28,

iv) a sequence complementary to any one of i) to iii).

47. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:34;

ii) a sequence of nucleotides as provided in SEQ ID NO:35;

iii) a sequence encoding a polypeptide according to claim 29;

iv) a sequence of nucleotides which is at least 70% identical to SEQ ID NO:34 or SEQ ID NO:35; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:52.

48. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:36;

ii) a sequence of nucleotides as provided in SEQ ID NO:37;

iii) a sequence encoding a polypeptide according to claim 30;

iv) a sequence of nucleotides which is at least 55% identical to SEQ ID NO:36 or SEQ ID NO:37; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:53.

49. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:38;

ii) a sequence encoding a polypeptide according to claim 31;

iii) a sequence of nucleotides which is at least 65% identical to SEQ ID NO:38; and

iv) a sequence which hybridizes to any one of i) to iii) under high stringency conditions.

50. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:39;

ii) a sequence of nucleotides as provided in SEQ ID NO:40;

iii) a sequence encoding a polypeptide according to claim 32;

iv) a sequence of nucleotides which is at least 95% identical to SEQ ID NO:39 or SEQ ID NO:40; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:54.

51. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:41;

ii) a sequence of nucleotides as provided in SEQ ID NO:42;

iii) a sequence encoding a polypeptide according to claim 33;

iv) a sequence of nucleotides which is at least 50% identical to SEQ ID NO:41 or SEQ ID NO:42; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:55.

52. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:43;

ii) a sequence of nucleotides as provided in SEQ ID NO:44;

iii) a sequence encoding a polypeptide according to claim 34;

iv) a sequence of nucleotides which is at least 65% identical to SEQ ID NO:43 or SEQ ID NO:44; and

v) a sequence which hybridizes to any one of i) to iv) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:56.

53. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of:

i) a sequence of nucleotides as provided in SEQ ID NO:45;

ii) a sequence encoding a polypeptide according to claim 35;

iii) a sequence of nucleotides which is at least 50% identical to SEQ ID NO:45; and

iv) a sequence which hybridizes to any one of i) to iii) under high stringency conditions,

wherein the polynucleotide does not comprise a sequence of nucleotides as provided in SEQ ID NO:57.

54. A catalytic polynucleotide capable of cleaving a polynucleotide according to any one of claims 38 to 53.

55. The catalytic polynucleotide of claim 54 which is a ribozyme.

56. An oligonucleotide which comprises at least 19 contiguous nucleotides of a polynucleotide according to any one of claims 38 to 53.

57. A double stranded RNA (dsRNA) molecule comprising an oligonucleotide according to claim 56, wherein the portion of the molecule that is double stranded is at least 19 basepairs in length and comprises said oligonucleotide.

58. The dsRNA molecule of claim 57 which is expressed from a single promoter, wherein the strands of the double stranded portion are linked by a single stranded portion.

59. A vector comprising or encoding the polynucleotide according to any one of claims 38 to 53.

60. The vector of claim 59 wherein the polynucleotide is operably linked to an ovule or fibre specific promoter.

61. A vector comprising or encoding oligonucleotide of claim 56 or the dsRNA molecule of claim 57.

62. A host cell comprising the vector according to any one of claims 59 to 61.

63. A transgenic plant, the plant having been transformed with polynucleotide according to any one of claims 38 to 53 or the oligonucleotide of claim 56.

64. The transgenic plant of claim 63 wherein the polynucleotide is capable of expression to produce a polypeptide according to any one of claims 19 to 37.

65. A transgenic plant, the plant having been transformed such that it produces a catalytic polynucleotide of claim 54 or claim 55, or a dsRNA molecule of claim 57 or claim 58.

66. The transgenic plant of claim 63 or claim 65, wherein the polynucleotide, catalytic polynucleotide or dsRNA down-regulates the production of a polypeptide according to any one of claims 19 to 37 which is endogenously produced by the plant.

67. A substantially purified antibody, or fragment thereof, that specifically binds a polypeptide according to any one of claims 19 to 37.

68. A method of breeding a fibre producing plant, the method comprising performing a method according to any one of claims 11 to 18.

69. A method of selecting from a breeding population a fibre producing plant with altered fibre initiation and/or elongation potential, the method comprising;

i) crossing two plants which have differing potential to produce fibre,

ii) performing a method according to any one of claims 11 to 18 on progeny plants,

iii) selecting a progeny plant with altered fibre initiation and/or elongation potential when compared to a parent plant.

70. A plant produced by the method of claim 68 or claim 69.

71. Seed of a plant of any one of claims 63 to 66 or claim 70.

72. Fibre of a plant of any one of claims 63 to 66 or claim 70.

73. A method of identifying an agent which alters fibre initiation and/or elongation of a fibre producing plant, the method comprising

a) exposing a polypeptide which is at least 50% identical to any one of SEQ ID NO's:1 to 16 to a candidate agent, and

b) assessing the ability of the candidate agent to modulate the activity of the polypeptide.

74. A method of identifying an agent which alters fibre initiation and/or elongation of a fibre producing plant, the method comprising

a) exposing a polypeptide which is at least 50% identical to any one of SEQ ID NO's:1 to 16 to a binding partner which binds the polypeptide, and a candidate agent, and

b) assessing the ability of the candidate agent to compete with the binding partner for binding to the polypeptide.

75. The method of claim 74, wherein the binding partner is detectably labeled.

76. A method of identifying an agent which alters fibre initiation and/or elongation of a fibre producing plant, the method comprising

a) exposing a polynucleotide encoding a polypeptide which is at least 50% identical to any one of SEQ ID NO's:1 to 16 to a candidate agent under conditions which allow expression of the polynucleotide, and

b) assessing the ability of the candidate agent to modulate levels of polypeptide produced by the polynucleotide.

77. A method of identifying an agent which alters fibre initiation and/or elongation of a fibre producing plant, the method comprising

a) exposing a polynucleotide which is at least 50% identical to any one of SEQ ID NO's:17 to 45 to a candidate agent, and

b) assessing the ability of the candidate agent to hybridize and/or cleave the polynucleotide.

SEQUENCE LISTING

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His Leu Arg Ile Glu Asn Ala Arg Leu Arg Glu Glu Ile Asp Arg Ile
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His Leu Ser Ser His Leu His Ser Arg Ser Ala Asp Leu Gly Ala Ser
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                245                 250                 255

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            260                 265                 270

Asn Tyr Phe Ala Arg Tyr Cys Lys Gln His Ile Asp Gly Thr Trp Ala
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Val Val Asp Val Ser Leu Asp Asn Leu Arg Pro Asn Pro Met Ser Ser
    290                 295                 300

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Lys Arg Trp Val Ala Thr Leu Asp Arg Gln Cys Glu Arg Leu Ala Ser
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    530                 535                 540

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545                 550                 555                 560

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                565                 570                 575

Gly Ser Leu Leu Thr Val Ala Phe Gln Ile Leu Val Asp Ser Val Pro
            580                 585                 590

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Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp
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Ile Lys Arg Gly Lys Phe Ser Leu Gln Glu Glu Gln Thr Ile Ile Gln
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Leu His Ala Leu Leu Gly Asn Arg Trp Ser Ala Ile Ala Thr His Leu
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Pro Lys Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Leu
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Met Lys Arg Leu Thr Lys Met Gly Ile Asp Pro Val Thr His Lys Pro
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115 120 125

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Pro Arg Pro Thr Leu Ser Lys Ser Val Gly Asn Gly Val Glu Phe Leu
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Asn Arg His Leu Ser Ala Lys Leu Phe His Asp Lys Glu Ser Met His
                165   170   175

Pro Leu Leu Glu Phe Leu Arg Val His Cys His Lys Gly Lys Asn Met
            180   185   190

Met Leu Asn Asp Arg Ile Gln Asn Leu Asn Ala Leu Gln His Val Leu
        195   200   205

Arg Lys Ala Glu Glu Tyr Leu Gly Thr Leu Pro Pro Glu Thr Pro Cys
    210   215   220

Ala Gly Phe Glu His Arg Phe Gln Glu Ile Gly Leu Glu Arg Gly Trp

225 230 235 240

Gly Asp Thr Ala Gln Arg Val Leu Glu Met Ile Gln Leu Leu Leu Asp
245 250 255

Leu Leu Glu Ala Pro Asp Pro Cys Thr Leu Glu Lys Phe Leu Gly Arg
260 265 270

Ile Pro Met Val Phe Asn Val Val Ile Leu Thr Pro His Gly Tyr Phe
275 280 285

Ala Gln Asp Asn Val Leu Gly Tyr Pro Asp Thr Gly Gly Gln Val Val
290 295 300

Tyr Ile Leu Asp Gln Val Arg Ala Leu Glu Asn Glu Met Leu Leu Arg
305 310 315 320

Ile Lys Gln Gln Gly Leu Asn Ile Thr Pro Arg Ile Leu Ile Ile Thr
325 330 335

Arg Leu Leu Pro Asp Ala Val Gly Thr Thr Cys Gly Gln Arg Leu Glu
340 345 350

Lys Val Tyr Gly Thr Glu Tyr Ser Asp Ile Leu Arg Val Pro Phe Arg
355 360 365

Thr Glu Lys Gly Ile Val Arg Lys Trp Ile Ser Arg Phe Glu Val Trp
370 375 380

Pro Tyr Leu Glu Thr Tyr Thr Glu Asp Val Ala His Glu Ile Ser Lys
385 390 395 400

Glu Leu Gln Gly Lys Pro Asp Leu Ile Ile Gly Asn Tyr Ser Asp Gly
405 410 415

Asn Ile Val Ala Ser Leu Leu Ala His Lys Leu Gly Val Thr Gln Cys
420 425 430

Thr Ile Ala His Ala Leu Glu Lys Thr Lys Tyr Pro Asp Ser Asp Ile
435 440 445

Tyr Trp Lys Lys Leu Glu Asp Lys Tyr His Phe Ser Cys Gln Phe Thr
450 455 460

Ala Asp Leu Phe Ala Met Asn His Thr Asp Phe Ile Ile Thr Ser Thr
465 470 475 480

Phe Gln Glu Ile Ala Gly Ser Lys Asp Thr Val Gly Gln Tyr Glu Ser
485 490 495

His Thr Ala Phe Thr Leu Pro Gly Leu Tyr Arg Val Val His Gly Ile
500 505 510

Asp Val Phe Asp Pro Lys Phe Asn Ile Val Ser Pro Gly Ala Asp Met
515 520 525

Glu Ile Tyr Phe Pro Tyr Thr Glu Glu Lys Arg Arg Leu Lys His Phe
530 535 540

His Thr Glu Ile Glu Asp Leu Leu Tyr Ser Lys Val Glu Asn Glu Glu
545 550 555 560

His Leu Cys Val Leu Asn Asp Arg Asn Lys Pro Ile Leu Phe Thr Met
565 570 575

Ala Arg Leu Asp Arg Val Lys Asn Leu Thr Gly Leu Val Glu Trp Tyr
580 585 590

Gly Lys Asn Ala Lys Leu Arg Glu Leu Ala Asn Leu Val Val Val Gly
595 600 605

Gly Asp Arg Arg Lys Glu Ser Lys Asp Leu Glu Glu Lys Ala Glu Met
610 615 620

Lys Lys Met Phe Glu Leu Ile Glu Lys Tyr Asn Leu Asn Gly Gln Phe
625 630 635 640

Arg Trp Ile Ser Ser Gln Met Asn Arg Ile Arg Asn Gly Glu Leu Tyr
645 650 655

Arg Tyr Ile Cys Asp Thr Lys Gly Ala Phe Val Gln Pro Ala Leu Tyr
660 665 670

Glu Ala Phe Gly Leu Thr Val Val Glu Ala Met Thr Cys Gly Leu Pro
675 680 685

Thr Phe Ala Thr Cys Asn Gly Gly Pro Ala Glu Ile Ile Val His Gly
690 695 700

Lys Ser Gly Phe Asn Ile Asp Pro Tyr His Gly Asp Gln Ala Ala Asp
705 710 715 720

Ile Leu Val Asp Phe Phe Glu Lys Cys Lys Lys Asp Pro Ser His Trp
725 730 735

Asp Lys Ile Ser Gln Gly Gly Leu Lys Arg Ile Glu Glu Lys Tyr Thr
740 745 750

Trp Lys Ile Tyr Ser Glu Arg Leu Leu Thr Leu Thr Gly Val Tyr Gly
755 760 765

Phe Trp Lys His Val Ser Asn Leu Glu Arg Arg Glu Ser Arg Arg Tyr
770 775 780

Leu Glu Met Phe Tyr Ala Leu Lys Tyr Arg Lys Leu Ala Glu Ser Val
785 790 795 800

Pro Leu Ala Glu Glu
805

<210> 10
<211> 195
<212> PRT
<213> Gossypium hirsutum

<400> 10

Met Glu Arg Gly Phe Ile Val Leu Ala Leu Thr Val Val Phe Ala Ala
1 5 10 15

Thr Val Val Thr Ala Ala Asp Glu Ser Gly Leu Ala Asn Glu Cys Ser
20 25 30

Lys Asp Phe Gln Ser Val Met Thr Cys Leu Ser Phe Ala Gln Gly Lys
35 40 45
Ala Ala Ser Pro Ser Lys Glu Cys Cys Asn Ser Val Ala Gly Ile Lys
50 55 60

Glu Asn Lys Pro Lys Cys Leu Cys Tyr Ile Leu Gln Gln Thr Gln Thr
65 70 75 80

Ser Gly Ala Gln Asn Leu Lys Ser Leu Gly Val Gln Glu Asp Lys Leu
85 90 95

Phe Gln Leu Pro Ser Ala Cys Gln Leu Lys Asn Ala Ser Val Ser Asp
100 105 110

Cys Pro Lys Leu Leu Gly Leu Ser Pro Ser Ser Pro Asp Ala Ala Ile
115 120 125

Phe Thr Asn Ser Ser Ser Lys Ala Thr Thr Pro Ser Thr Ser Thr Thr
130 135 140

Thr Ala Thr Pro Ser Ser Ala Ala Asp Lys Thr Asp Ser Lys Ser Ser
145 150 155 160

Gly Ile Lys Leu Gly Pro His Phe Val Gly Ser Thr Ala Ala Leu Leu
165 170 175

Val Ala Thr Ala Ala Val Phe Phe Leu Val Phe Pro Ala Gly Phe Ala
180 185 190

Ser Ile Val
195

<210> 11
<211> 123
<212> PRT
<213> Gossypium hirsutum

<400> 11

Met Ala Ser Ser Gly Val Leu Lys Leu Val Ser Met Ile Leu Met Val
1 5 10 15

Cys Met Thr Met Met Ser Ala Pro Lys Ala Ala Lys Ala Ala Ile Thr
20 25 30

Cys Ser Asp Val Val Asn His Leu Ile Pro Cys Leu Ser Tyr Val Gln
35 40 45

Asn Gly Gly Thr Pro Ala Ala Ala Cys Cys Ser Gly Val Lys Ala Leu
50 55 60

Tyr Gly Glu Val Gln Thr Ser Pro Asp Arg Gln Asn Val Cys Lys Cys
65 70 75 80

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Ile Lys Ser Ala Val Asn Gly Ile Pro Tyr Thr Ser Asn Asn Leu Asn
                85                  90                  95

Leu Ala Ala Gly Leu Pro Ala Lys Cys Gly Leu Gln Leu Pro Tyr Ser
            100                 105                 110

Ile Ser Pro Ser Thr Asp Cys Asn Lys Val Gln
        115                 120


<210>  12
<211>  282
<212>  PRT
<213>  Gossypium hirsutum

<400>  12

Pro Arg Val Arg Pro Arg Val Arg Ala His Leu Pro Lys Arg Thr Asp
1               5                   10                  15

Asn Glu Ile Lys Asn Tyr Trp Asn Thr Gln Leu Lys Lys Arg Leu Thr
            20                  25                  30

Thr Ile Gly Ile Asp Pro Ala Thr His Arg Pro Lys Thr Asp Thr Leu
        35                  40                  45

Gly Ser Thr Pro Lys Asp Ala Ala Asn Leu Ser His Met Ala Gln Trp
    50                  55                  60

Glu Ser Ala Arg Leu Glu Ala Glu Ala Arg Leu Val Arg Glu Ser Lys
65                  70                  75                  80

Arg Val Ser Asn Pro Ser Gln Asn Gln Phe Arg Phe Thr Ser Ser Ser
                85                  90                  95

Ala Pro Pro Leu Val Ser Lys Ile Asp Val Gly Leu Ala His Ala Thr
            100                 105                 110

Lys Pro Gln Cys Leu Asp Val Leu Lys Ala Trp Gln Arg Val Val Thr
        115                 120                 125

Gly Leu Phe Thr Phe Asn Thr Asp Asn Leu Gln Ser Pro Thr Ser Thr
    130                 135                 140

Ser Ser Phe Thr Glu Asn Thr Leu Pro Ile Ser Ser Val Gly Phe Ile
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145 150 155 160
Asp Ser Phe Val Gly Asn Ser Asn Asn Ser Cys Cys Gly Asn Asn Trp
165 170 175

Glu Cys Val Glu Lys Ser Ser Gln Val Ala Glu Leu Gln Glu Arg Leu
180 185 190

Asp Asn Ser Met Gly Leu His Asp Ile Leu Asp Leu Ser Ser Glu Asp
195 200 205

Val Trp Phe Gln Gly Ser Tyr Arg Ala Glu Asn Met Met Glu Gly Tyr
210 215 220

Ser Asp Thr Leu Met Val Cys Asp Ser Gly Asp His Pro Lys Ser Leu
225 230 235 240

Ser Met Glu Pro Arg Gln Asn Phe Asn Val Gly Thr Ser Asn Ala Ser
245 250 255

Ser Phe Glu Glu Asn Lys Asn Tyr Trp Asn Asn Ile Leu Asn Phe Ala
260 265 270

Asn Ala Ser Pro Ser Gly Ser Ser Val Phe
275 280

<210> 13
<211> 177
<212> PRT
<213> Gossypium hirsutum

<400> 13

Met Lys Val Leu Ser Pro Ile Leu Ala Cys Leu Ala Leu Ala Val Val
1 5 10 15

Ala Ser His Ala Ala Leu Ser Pro Glu Gln Tyr Trp Ser Tyr Lys Leu
20 25 30

Pro Asn Thr Pro Met Pro Lys Ala Val Lys Glu Ile Leu His Pro Glu
35 40 45

Leu Met Glu Glu Lys Ser Thr Ser Val Asn Val Gly Gly Gly Gly Val
50 55 60

Asn Val Asn Thr Gly Lys Gly Lys Pro Ala Gly Gly Thr His Val Asn

65 70 75 80

Val Gly Arg Lys Gly Val Gly Val Asn Thr Gly Lys Pro Gly Gly Gly
85 90 95

Thr His Val Asn Val Gly Gly Lys Gly Val Gly Val Asn Thr Gly Lys
100 105 110

Pro Gly Gly Gly Thr His Val Asn Val Gly Gly Lys Gly Gly Gly Val
115 120 125

Ser Val His Thr Gly His Lys Gly Lys Pro Val Asn Val Asn Val Ser
130 135 140

Pro Phe Leu Tyr Gln Tyr Ala Ala Ser Glu Thr Gln Ile His Asp Asp
145 150 155 160

Pro Asn Val Ala Leu Phe Phe Leu Glu Lys Asp Leu His Pro Gly Gln
165 170 175

Gln

<210> 14
<211> 282
<212> PRT
<213> Gossypium hirsutum

<400> 14

Leu Ser Glu Ser Lys Glu Met Val Phe Gln Phe Asn Phe Pro Val Leu
1 5 10 15

Leu Leu Cys Leu Met Phe Leu Met Cys Gly Arg Gly Asn Ala Val Arg
20 25 30

Asp Leu Glu Gly Lys His Asp Phe Glu Ser His Gly Arg Asp Asp Glu
35 40 45

Val Glu Ser Leu Asp Asp Lys Tyr Val Ser Ala Tyr Phe His Gln Thr
50 55 60

Phe Asp Ser Ala Asn His Phe Asp Gly Gly Asp Glu Val Lys Asn Leu
65 70 75 80

Glu Asp Lys Tyr Ser Thr Ala Tyr Phe His Lys Ser Leu Asp Ser Gly

85 90 95

Asn His Gly Arg Asp Asp Lys Ala Lys Ile Leu Glu Asp Lys Tyr Ala
100 105 110

Thr Ala Tyr Phe His Lys Thr Ser Val Phe Glu Asn His Gly Glu Gly
115 120 125

Asp Lys Leu Lys Ser Leu Glu Asp Lys Tyr Ser Ala Ala Tyr Phe His
130 135 140

Asn Thr Gln Ser Ser Lys Met Met Lys Asp His Asn Met Glu His His
145 150 155 160

His His Tyr His Asn His Val Glu Ser Ala Glu Ile Gly Leu Phe Thr
165 170 175

Ile Asp Glu Leu His Thr Phe Asn Val Gly Lys Lys Leu Pro Ile Phe
180 185 190

Phe Pro Ile Lys Asn His Ser Leu Tyr Pro Pro Leu Leu Pro Lys Gln
195 200 205

Ile Ala Asp Thr Ile Pro Phe Ser Ser Phe Gln Val Ser Asn Ile Leu
210 215 220

Arg Phe Phe Ser Val Ser Pro Asp Ser Pro Lys Gly Lys Ser Cys Ser
225 230 235 240

Arg Tyr Leu Arg Lys Met Arg Thr Arg Ser Ser Ala Arg Gly Arg Pro
245 250 255

Lys Ile Trp Ala Thr Ser Leu Lys Ser Leu His Gly Phe Leu Ser Met
260 265 270

His Leu Gly Pro Met Leu Ile Ser Ser Ser
275 280

<210> 15
<211> 55
<212> PRT
<213> Gossypium hirsutum

<400> 15

Lys Trp Glu Ala Gly Gln Ser Gln Cys Met Val Val Leu Val Phe Thr

1 5 10 15

Gln Ile Ser Leu Val Lys Gly Lys Arg Lys Leu Cys Tyr Ser Ser Ile
20 25 30

Val Ala Leu Ile Leu Glu Ser Val Leu Phe Val Leu Thr Phe Pro Ala
35 40 45

Leu Thr Asp Met Asn Leu Tyr
50 55

<210> 16
<211> 235
<212> PRT
<213> Gossypium hirsutum

<400> 16

Met Pro Arg Thr Arg Arg Phe Asn Pro Pro Ser Ile Thr Ser Arg Thr
1 5 10 15

Leu Gly His His Val Tyr Lys Asp Asp Asn Pro Ile Val Tyr Gly Thr
20 25 30

Met Gln Ala Tyr Leu Lys Asp Ala Arg Glu Arg Leu Phe Asn Thr Ala
35 40 45

Arg Thr Ala Glu Lys Leu Gly Ile His Met Gly Phe Lys Leu Val Arg
50 55 60

Gly Ala Tyr Met Ser Ser Glu Thr Lys Leu Ala Ser Ser Leu Gly Phe
65 70 75 80

Asp Ser Pro Val His Asn Thr Ile Gln Asp Thr His Ala Cys Phe Asn
85 90 95

Asp Cys Ala Ser Phe Met Ile Glu Lys Ile Ala Asp Gly Tyr Gly Gly
100 105 110

Leu Val Leu Ala Thr His Asn Leu Glu Ser Gly Lys Leu Ala Ala Ser
115 120 125

Lys Ala Arg Asn Leu Gly Ile Glu Lys Gly Asn Gln Lys Leu Glu Phe
130 135 140

Ala Gln Leu Tyr Gly Met Ser Glu Ala Leu Ser Ile Gly Leu Arg Asn

145 150 155 160

Ala Gly Phe Gln Val Ser Lys Tyr Leu Pro Tyr Gly Pro Val Asp Met
165 170 175

Val Met Pro Tyr Leu Leu Arg Arg Ala Glu Glu Asn Arg Gly Leu Leu
180 185 190

Ser Thr Ser Ser Leu Asp Arg Thr Leu Met Gly Lys Glu Leu Lys Arg
195 200 205

Arg Leu Lys Ser Leu Gln Phe Ala Lys Pro Glu Met Ala Ala Ser Ala
210 215 220
Ala Gly Ser Met Lys Ile Glu Ile Gly Thr Pro
225 230 235

<210> 17
<211> 2207
<212> DNA
<213> Gossypium hirsutum

<400> 17
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gctctcagca atgctacatg ccccagctgt ggaggcccag ctgcccttgg agagatgtca 180
tttgatgagc aacatttgag aatagaaaat gctcggttaa gggaagagat tgataggata 240
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ccaatgtcaa gtgtagagag gccctcaggt tgcttgatcc agaattgcca aatggatacc 960

tctaaggtta tatgggtcga gcatgtagaa gtggatgata gagctgtcca caacatatac 1020

agaccagtag ttaattccgg tctagctttt ggagcaaaac gttgggtggc tacgttggat 1080

cgacagtgtg agcgtctagc aagttcaatg gccagtaaca ttccagcagg gggtctatgc 1140

gttataacaa gcccagaagg gaggaaaagt atgttgaagt tggcagagag gatggtgact 1200

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gatttcctaa gggatgagaa ctctagaagt gagtgggata tcctatcaaa tggtggccta 1440

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cgcgtaaata gtgcaaactc tagccaaagc aacatgttga tacttcaaga gagctgcact 1560
gatgctaaag ggtcctacgt gatatatgcc ccggtcaata ttgttgcaat gaacatcgtc 1620

ttaagtggcg gggacccgga ttatgtcgca ctattgccat ccggtttcgc aattcttccc 1680

gatggtccag gagttaatgg aggagggatc ctcgaaatcg gctcgggtgg ctctctcctt 1740

accgttgctt tccagatttt ggttgattca gttcccacag caaagctttc tcttggatca 1800

gtggcgactg tcaacagtct aattaaatgc acggttgaaa ggatcaaggc tgccgtaaag 1860

tgcaataatg cttgaccaaa catgatataa aaaaaggaaa cgagaagaaa aggtgtttgt 1920

ccgaaaacaa atttaacgat tgaagaagtc aagagcgcac ctttcaattc atcctttgcg 1980

gtcatggtgt tctgtaagaa ggcaaaatca tcaagcctgc aaggatagta ggttcgggaa 2040

ttgactttgc caacgagatt ctaatattag atatgttggg agaactcccc attttgtgta 2100

ggctaagagt tcaatgtagg agtggacttt atactagtct aatttctttc tggtttcatg 2160

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<210> 18
<211> 1872
<212> DNA
<213> Gossypium hirsutum

<400> 18
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gctctcagca atgctacatg ccccagctgt ggaggcccag ctgcccttgg agagatgtca 180

tttgatgagc aacatttgag aatagaaaat gctcggttaa gggaagagat tgataggata 240

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tgcaataatg ct 1872

<210> 19
<211> 1180
<212> DNA
<213> Gossypium hirsutum

<400> 19
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taactacttg agacctgata tcaaaagagg aaagttcagt ttacaagaag aacagaccat 300

tattcaactc catgcccttc ttggaaacag gtggtctgcc atagctactc atttgccgaa 360

aagaacagac aatgagatca agaactactg gaacacacat ctaatgaaaa ggctaaccaa 420

aatggggatc gatcctgtca cccacaagcc taaaaccgat gcactcggct ccaccactgg 480

taaccctaaa gatgctgcta accttagtca catggctcaa tgggagagtg ctcgtttaga 540

agctgaagct agactggttc gtgagtccaa gctagttcct tcaaaccctc ctcaaagcaa 600

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atggtttcaa gagttgtttg gatttaatgg tttatgattc tgcagaagga ttcatcaaag 1020

gaaagaaagc tatctggttt catctttgaa gttcacttaa gtgtaggatt tttattcaca 1080

agtgccttca catattacca ttaactgtaa taataaacct tcaaattaat aaattaaaaa 1140

actcacaagg gtttttggcc aaaaaaaaaa aaaaaaaaaa 1180

<210> 20
<211> 927
<212> DNA
<213> Gossypium hirsutum

<400> 20
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gaagatcaaa agctcttagc ttacattgaa caacatggcc atggaagctg gcgtgccttg 120

ccttcaaaag ctgggcttca aagatgtgga aagagttgca gactgagatg gattaactac 180

ttgagacctg atatcaaaag aggaaagttc agtttacaag aagaacagac cattattcaa 240

ctccatgccc ttcttggaàa caggtggtct gccatagcta ctcatttgcc gaaaagaaca 300

gacaatgaga tcaagaacta ctggaacaca catctaatga aaaggctaac caaaatgggg 360

atcgatcctg tcàcccacaa gcctaaaacc gatgcactcg gctccaccac tggtaaccct 420

aaagatgctg ctaaccttag tcacatggct caatgggaga gtgctcgttt agaagctgaa 480

gctagactgg ttcgtgagtc caagctagtt ccttcaaacc ctcctcaaag caaccatttc 540

actgccgttg cgccttcgcc gactccggca actagaccgc aatgcctcga cgtactcaaa 600

gcatggcaag gtgtcgtctg cgggttattc actttcaaca tggacaataa caacttacag 660

tcccctacgt caacgttgaa cttcatggag aacaccacaa cattgcccat gtcàtcatca 720

tcgtctgtta atggaatgtt taatgaaaac tttggttgga actcatcgat taatccatgt 780

gaaagtgggg ataatttgaa agttgaatat ggcagtgatc aaattccaga gttaaaggaa 840

agattggatc atccaatgga attgcatgaa atggactatt cttcagaggg tacatggttt 900

caagagttgt ttggatttaa tggttta 927

<210> 21
<211> 600
<212> DNA
<213> Gossypium hirsutum
<400> 21

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cctctgtatt ggctactgca accatgatgc acgtcataga ccaagttgag cttttcaatc 120

ccattgacta ccaaaatcag ctgctgagtg ttcttaaaat tagcaaggaa aaagtaaacg 180

attgttacaa gctcatcctt gatgtatcaa caagacccca ggcccaaggc aatggtggtg 240

catgtaagag gaaggtggag gagagggttc ctagcagccc tagtggagtg attgatgctg 300

catttggcag tgatagctcg agcgattctt ggggcacggt gtccttatcg cctgagcagc 360

agccaccttt taagaagagc agagcccaag agcaagtaat gcgtttgcca tcactcaacc 420

gagtctttgt agacattgtt ggcagccctt cttaattata tctcccttct ctctctccct 480

cgctctctcc atctctttct ttgtcccaaa aagatctata tttattatgc ttatgttcac 540

ttttggttca aggaatcaaa tgttaagtta aaaaaatgaa aaaaacaaag taaaagctgc 600

<210> 22
<211> 452

<212> DNA
<213> Gossypium hirsutum

<400> 22
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ccattgacta ccaaaatcag ctgctgagtg ttcttaaaat tagcaaggaa aaagtaaacg 180

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<212> DNA
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<211> 727
<212> DNA
<213> Gossypium hirsutum

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catcaat 727

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<211> 562
<212> DNA
<213> Gossypium hirsutum

<220>
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<212> DNA
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<213> Gossypium hirsutum

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<211> 571
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<213> Gossypium hirsutum

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<210> 32
<211> 2611
<212> DNA
<213> Gossypium hirsutum

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<213> Gossypium hirsutum

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<210> 36
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gcggtacacc cgctgctgca tgctgcagtg gggtaaaagc actctacggc gaggttcaga 240

cctccccgga ccgccaaaac gtgtgcaagt gcatcaaatc ggcggtgaac ggaattccgt 300

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cttacagcat cagcccctcc actgactgca acaaggtgca gtgaggttga tgatgatgat 420

atggaaggag tggaagaagg ttccagctca gctagataaa gtagctagct aaggttaaat 480

aagctgtgtt ggtgtgttgt tttttagaaa attccatata taatcgggga aagaaaaaaa 540

aatagaaaat gtactttgta actgtatttc gtatgtgata tatataatgt atcgtaatct 600

ttaatttttt 610

<210> 37
<211> 369
<212> DNA
<213> Gossypium hirsutum

<400> 37
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atgagtgcac ccaaggcagc caaagccgcc atcacgtgca gcgacgtggt gaaccacttg 120

atcccgtgct tgtcctacgt acaaaacggc ggtacacccg ctgctgcatg ctgcagtggg 180

gtaaaagcac tctacggcga ggttcagacc tccccggacc gccaaaacgt gtgcaagtgc 240

atcaaatcgg cggtgaacgg aattccgtac accagcaata acctcaatct cgcagccggc 300

ctacctgcta aatgtggtct ccaactccct tacagcatca gcccctccac tgactgcaac 360

aaggtgcag 369

<210> 38
<211> 886
<212> DNA
<213> Gossypium hirsutum

<400> 38
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aactactgga atacacagtt gaagaaaagg ttgacgacga tagggatcga ccctgcaact 120

cacaggccta aaaccgatac cctcggttct actcccaagg atgccgctaa ccttagccac 180

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cgagtttcaa acccttcgca aaaccaattt aggttcacgt cttcatcggc tcctccactg 300

gtaagcaaaa ttgatgttgg tttggctcat gctactaaac cgcaatgcct cgatgtactc 360

aaagcttggc aacgtgtagt cactggattg ttcactttca acactgacaa cctccaatct 420

ccaacatcga cgtcgagctt cacggaaaac acgttaccaa tctcatctgt cgggttcatt 480

gacagctttg tggggaactc aaataacagc tgttgcggaa ataattggga atgtgtggag 540

aaatcgagcc aagttgctga attacaggaa agattggata actcaatggg gttgcatgac 600

atattggatc tctcctcaga agatgtatgg tttcaaggct catacagggc ggaaaatatg 660

atggaagggt attcggacac gttaatggtt tgtgattctg gggatcatcc gaagagtttg 720

tcaatggagc ctagacaaaa ctttaatgtt ggaacaagta atgctagtag tttcgaagaa 780

aacaagaatt actggaacaa catccttaat tttgcgaatg cttccccttc tggttcttct 840

gtcttttgag attaattgtt aagatttgaa ataaataaaa atatat 886

<210> 39
<211> 1353
<212> DNA
<213> Gossypium hirsutum

<400> 39
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ctctcacccg agcaatattg gagctataag ctgccaaata ctccaatgcc aaaggctgtc 180

aaagaaattc tacatccaga actgatggag gagaaaagta cctctgtaaa tgtaggaggt 240

ggtggtgtaa acgtcaacac aggaaaaggg aagccagcgg gtggcactca tgtgaacgtt 300

gggcgcaaag gagttggagt gaacacggga aagccagggg gtggcactca tgtgaatgtt 360

ggaggcaaag gagttggggt gaacactgga aagccaggag gtggcaccca tgtgaacgtt 420

ggaggcaaag gtggaggagt atctgtacac accggacaca agggaaagcc agtaaatgtt 480

aatgtgagtc cgtttcttta ccaatatgca gccagtgaaa ctcaaatcca tgacgatccg 540

aatgtggctc ttttctttct ggaaaaggat ttacaccccg ggcaacaatg agcctgcatt 600

tcacttgaaa atacagagaa atccctttct taccttatca aactgccaaa aaaatccgtt 660

ttcatttacg aagttgccag aattttcaca agttttcagt gaacctggat cagtgaaggc 720

agagatgatg aagaacccat taaggagtgc gaacagccag cgattgaagg agaggaaaaa 780

tattgtgcac cctcactgga gtcaatgatt gactacagca tttccaaact agggaaagtt 840

gatcaggcag tctcaacaga agtggaaaaa caaaccccaa cgcacaagta tacaataaca 900

gctggagtgc agaagatgac aaatgacaaa gctgtagtgt gccacaagca gaattatgca 960

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catttccttc ctcgggatca cattgtttgg gtccccaagt aaaagtcctg aagagtagac 1200

tcatacacta tagtttcatc atagggtgca ttaaaacagc ttaaagcaat ctccagtttg 1260

ttctataata atatacccac gagtttagtc atgtaaaatc tatccatgaa tcatgttctt 1320

agtaatggat aaaatgatag tactttctgt atc 1353

<210> 40
<211> 1122
<212> DNA
<213> Gossypium hirsutum

<400> 40
atgaaggttc tctccccaat tcttgcttgc ctagcgcttg ctgtggtggc aagtcatgct 60

gctctctcac ccgagcaata ttggagctat aagctgccaa atactccaat gccaaaggct 120

gtcaaagaaa ttctacatcc agaactgatg gaggagaaaa gtacctctgt aaatgtagga 180

ggtggtggtg taaacgtcaa cacaggaaaa gggaagccag cgggtggcac tcatgtgaac 240

gttgggcgca aaggagttgg agtgaacacg ggaaagccag gggtggcac tcatgtgaat 300

gttggaggca aaggagttgg ggtgaacact ggaaagccag gaggtggcac ccatgtgaac 360

gttggaggca aaggtggagg agtatctgta cacaccggac acaagggaaa gccagtaaat 420

gttaatgtga gtccgtttct ttaccaatat gcagccagtg aaactcaaat ccatgacgat 480

ccgaatgtgg ctcttttctt tctggaaaag gatttacacc ccgggcaaca atgagcctgc 540

atttcacttg aaaatacaga gaaatccctt tcttacctta tcaaactgcc aaaaaaatcc 600

gttttcattt acgaagttgc cagaattttc acaagttttc agtgaacctg gatcagtgaa 660

ggcagagatg atgaagaacc cattaaggag tgcgaacagc cagcgattga aggagaggaa 720

aaatattgtg caccctcact ggagtcaatg attgactaca gcatttccaa actagggaaa 780

gttgatcagg cagtctcaac agaagtggaa aaacaaaccc caacgcacaa gtatacaata 840

acagctggag tgcagaagat gacaaatgac aaagctgtag tgtgccacaa gcagaattat 900

gcatatgctg tcttctattg ccataaatga gaaacaacaa gggcttacat ggttccttta 960

gagggtgctg acggaacaaa agccaaagca gtagcagtct gtcacacaga tacatcagca 1020

tggaacccaa agcatttggc ttttcaagtc ctaaaagttg agccaggaac cattcctgtc 1080

tgccatttcc ttcctcggga tcacattgtt tgggtcccca ag 1122

<210> 41
<211> 1373
<212> DNA
<213> Gossypium hirsutum

<220>
<221> misc_feature
<222> (895)..(895)
<223> n = unknown

<220>
<221> misc_feature
<222> (911)..(911)
<223> n = unknown

<220>
<221> misc_feature
<222> (1270)..(1270)
<223> n = unknown

<220>
<221> misc_feature
<222> (1336)..(1336)
<223> n = unknown

<400> 41
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ttgaaagcca tggcagagac gacgaagtgg agagtttaga tgacaagtac gttagcgctt 180

actttcatca aacttttgat tctgcaaatc actttgatgg aggtgatgaa gtgaagaatt 240

tagaagacaa atattcaacg gcttacttcc acaaatcgtt agattctgga aaccatggca 300

gagatgacaa agcaaagata ttggaagaca agtatgctac tgcgtacttc cacaagactt 360

ctgtttttga aaaccatggt gaaggtgaca aattaaagag tttggaagat aaatattccg 420

cggcttactt tcacaacaca caatcttcca aaatgatgaa ggatcacaac atggaacatc 480

accaccatta ccataaccat gttgaaagtg cagagatagg cttgttcacc attgatgaac 540

tacatacctt taacgtaggg aagaaattac ccatcttttt cccaataaaa aaccactctc 600

tttaccctcc tttattgcct aaacaaattg ctgacaccat ccctttttca tctttccaag 660

tttctaatat tctacgattc ttctcagttt ctccggactc ccccaaaggc aaaagctgtt 720

caagatacct tcgcaaaatg cgaactcgga gcagcgcaag ggggagaccc aaaatctggg 780

ctacctcttt aaaatcttta catgggtttc taagcatgca tttgggcccc atgttgattt 840

caagttcata agccaaggca tccccccata ccaaccccac tctttcaaag ttacncagtt 900

ttagaatccc ntgaagagat tgaatctcca aagaaagtag catgtcatcc aatgccatat 960

ctttatgcag tttatttctg tcactttgat gccactgaga ttaaagcttt caaactccgt 1020

ttagttggtg atgttacggg agataaggtg gatgctgttg ttctttgcca tatggatact 1080

tcaggttgga gctctgatca tgtcgctttt cgcatgcttg gtattaagca aggaaacact 1140

gtttgccatg tattttctca aggtaatctt gtttggatta atcagccatc ggatatcgct 1200

gccggtgcca tataagtgtt gaactgttcg atgtagcact catttgccac tacgtatcga 1260

gaccttatcn caatataagt atttaagagc tagtcttatg ttcactaggt ttcatggtgt 1320

ttcgttaatg gtgtgncttt ctatctatat taagtatcaa gtaattaagc aat 1373

<210> 42
<211> 1212
<212> DNA
<213> Gossypium hirsutum

<220>
<221> misc_feature
<222> (895)..(895)
<223> n = unknown

<220>
<221> misc_feature
<222> (911)..(911)
<223> n = unknown

<400> 42
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ttatgttttt aatgtgtggc agaggcaatg cagtaaggga tttggaaggg aaacatgatt 120

ttgaaagcca tggcagagac gacgaagtgg agagtttaga tgacaagtac gttagcgctt 180

actttcatca aacttttgat tctgcaaatc actttgatgg aggtgatgaa gtgaagaatt 240

tagaagacaa atattcaacg gcttacttcc acaaatcgtt agattctgga aaccatggca 300

gagatgacaa agcaaagata ttggaagaca agtatgctac tgcgtacttc cacaagactt 360

ctgtttttga aaaccatggt gaaggtgaca aattaaagag tttggaagat aaatattccg 420

cggcttactt tcacaacaca caatcttcca aaatgatgaa ggatcacaac atggaacatc 480

accaccatta ccataaccat gttgaaagtg cagagatagg cttgttcacc attgatgaac 540

tacataccttt taacgtaggg aagaaattac ccatcttttt cccaataaaa aaccactctc 600

tttaccctcc tttattgcct aaacaaattg ctgacaccat ccctttttca tctttccaag 660

tttctaatat tctacgattc ttctcagttt ctccggactc ccccaaaggc aaaagctgtt 720

caagatacct tcgcaaaatg cgaactcgga gcagcgcaag ggggagaccc aaaatctggg 780

ctacctcttt aaaatcttta catgggtttc taagcatgca tttgggcccc atgttgattt 840

caagttcata agccaaggca tccccccata ccaaccccac tctttcaaag ttacncagtt 900

ttagaatccc ntgaagagat tgaatctcca aagaaagtag catgtcatcc aatgccatat 960

ctttatgcag tttatttctg tcactttgat gccactgaga ttaaagcttt caaactccgt 1020

ttagttggtg atgttacggg agataaggtg gatgctgttg ttctttgcca tatggatact 1080

tcaggttgga gctctgatca tgtcgctttt cgcatgcttg gtattaagca aggaaacact 1140

gtttgccatg tattttctca aggtaatctt gtttggatta atcagccatc ggatatcgct 1200

gccggtgcca ta 1212

<210> 43
<211> 1024
<212> DNA
<213> Gossypium hirsutum

<400> 43
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tgcatggtgg tgctggtgtt cacccaaatc tccctagtga aaggcaagag gaagctgtgc 120

tactcctcaa tcgttgcctt gatattggaa tctgtgcttt ttgttctaac ctttccggca 180

ttgactgaca tgaacttgta ttgagggaat ttgaacggat cctttgctta attccgggcg 240

tggatcggca cttacggata aagggacgga ggaaatggaa acttgcttta tggatggacc 300

gaacagacca tgcggtgctg tttcgggtaa acgacatgga agaatccgat atctcttgct 360
cgacttgaaa tggataaaac accacattca tctttgggtt ttgccggcgc cgattatttt 420

gcgaggaaac agggtgtgga gttggtggac aatgaatatt tcattacaga atacaatgtg 480

gggatgctta agttaacaaa agaagcacac tcaatcctgt actattaccg tatcctaacc 540

ctcaccacct gcggaggcag cgcagacatg gaaaatcgat tacgaatgaa ctggttacca 600

atctttctct acatcatata aacagtgggt cgagtcgcac catacaaaca atgtcattgc 660

tctgccgcta cttgcaccgg tggattaatg aacattatga ccggaaagat tggtgactcg 720

ccgctgattg gttcagagac ttatgcttgt gacttattgg ctgtttatgt accggtgaat 780

gtgaagccat tatgctaagc actttggcta cggaagtagc agcgtgatgg aatataaatg 840

gttgaatctt cctgaagctg tggatatgtg attaaactag actatgtgaa ggcaaagctg 900

gtctattgcc tgtcctatat gggaagtgct tggggctgaa tactactggt atgatatggt 960

tggctactga agatggatta tggaagttgt tgtctgcaaa ttgatgttag cttagatgct 1020

ggtc 1024

<210> 44
<211> 795
<212> DNA
<213> Gossypium hirsutum

<400> 44
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tgcatggtgg tgctggtgtt cacccaaatc tccctagtga aaggcaagag gaagctgtgc 120

tactcctcaa tcgttgcctt gatattggaa tctgtgcttt ttgttctaac ctttccggca 180

ttgactgaca tgaacttgta ttgagggaat ttgaacggat cctttgctta attccgggcg 240

tggatcggca cttacggata aagggacgga ggaaatggaa acttgcttta tggatggacc 300

gaacagacca tgcggtgctg tttcgggtaa acgacatgga agaatccgat atctcttgct 360

cgacttgaaa tggataaaac accacattca tctttgggtt ttgccggcgc cgattatttt 420

gcgaggaaac agggtgtgga gttggtggac aatgaatatt tcattacaga atacaatgtg 480

gggatgctta agttaacaaa agaagcacac tcaatcctgt actattaccg tatcctaacc 540

ctcaccacct gcggaggcag cgcagacatg gaaaatcgat tacgaatgaa ctggttacca 600

atctttctct acatcatata aacagtgggt cgagtcgcac catacaaaca atgtcattgc 660

tctgccgcta cttgcaccgg tggattaatg aacattatga ccggaaagat tggtgactcg 720

ccgctgattg gttcagagac ttatgcttgt gacttattgg ctgtttatgt accggtgaat 780

gtgaagccat tatgc 795

<210> 45
<211> 989
<212> DNA
<213> Gossypium hirsutum

<400> 45
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acaccaaaga ctgttaaaac tttgccaaaa tgcgcgcagt acaacgttcc tttaaccatt 120

gatgccgagg acacgtcgat tcaacccgcc atcgattact tcacgtactc tcggccatca 180

tgtatacaaa gatgataacc ccattgtcta cggcacgatg caagcttact tgaaagacgc 240

gagggagcgg ctgtttaaca cggcgaggac ggcggagaag ctggggattc atatggggtt 300

taagctggtg agaggcgctt acatgtcgag cgaaaccaag ttggcttctt ccttagggtt 360

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gtttatgatt gagaagattg ctgatgggta tggcggactc gttctcgcaa ctcataatct 480

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aaagcttgaa tttgcacagt tatatggaat gtcggaagcg ctgtcgattg gattgagaaa 600

cgcagggttt caagttagca aatacttacc ctatggacca gttgatatgg taatgccata 660

ccttttaagg agagccgaag aaaatagagg actcttatca acttcaagcc ttgatagaac 720

tctcatgggg aaggagttga agagaagatt aaagagcctg caatttgcga agccagagat 780

ggcagcttca gcagcaggta gcatgaagat agaaatagga acgccataaa tgaggttttg 840

attcatagat ggtttgggat gggcaatttt tgccaacaat gtagaattat gaaaaaaaaa 900

taacaatcat tgtaacgttt gggcatttgt cccatgtcaa ttattatttg cattagaaat 960

tgaatttttt tctttatttt tgaaaaaaa 989

<210> 46
<211> 410
<212> DNA
<213> Gossypium arboreum

<400> 46
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tttcaattca tcctttgcgg tcatggtgtt ttgtaagaag gcaaaatcac caagcctgca 180

aggatagtag gttcgggaat tgactttgcc aaagagattt taatattaga tatgttggga 240

gaactcccca ttttgtgtag gctaagagtt caatgtagga gtggacttta tactagtcta 300

atttcttttc agtttcatgt gttattgttg aagcattagt tattttggac ttattcctcc 360

attaacaaac atttgttaat ttctgcttaa aaaaaaaaaa aaaaaaaaaa 410

<210> 47
<211> 665
<212> DNA
<213> Gossypium arboreum

<220>
<221> misc_feature
<222> (19)..(19)
<223> n = unknown

<220>

<221> misc_feature
<222> (112)..(112)

<223> n = unknown

<400> 47
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tcccatggga gtttcttaag cgatgtgagc gtctcctcct ctgtgtaatc tntgattcaa 120

gatccatcca ttatcttccc tctgtattgg ctactgcaac catgatgcac gtcatagacc 180

aagttgagct tttcaatccc attgactacc aaaatcagct gctgagtgtt cttaaaatta 240

gcaaggaaaa agtaaacgat tgttacaagc tcatccttga tgtatcaaca agaccccagg 300

cccaaggcaa tggtggtgca tgtaagagga aggtggagga gagggttcct agcagcccta 360

gtggagtgat tgatgctgca tttggcagtg atagctcgaa cgattcgtgg ggcacggtgt 420

ccttatcgcc tgagcagcag ccacctttta agaagagcag agcccaagag caagtaatgc 480

gtttgccatc actcaaccga gtctttgtag acattgttgg cagcccttct taattatatc 540

tcccttctct ctctccctcg ctctctccat ctctttcttt gtcccaaaaa gatctatatt 600

tattatgctt atgttcactt ttggttcaag gaatcaaatg ttaagttaaa aaaaaaaaaa 660

aaaaa 665

<210> 48
<211> 626
<212> DNA
<213> Gossypium hirsutum

<220>
<221> misc_feature
<222> (581)..(581)
<223> n = unknown

<400> 48
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ctgaacaccc caaagatggc caaccacacc gttacctttc tccctaaact atccattgaa 120

gctattcaga cagtgactcc gatgaggata actgaaccac gacagactcg acaagtattg 180

gcaggggagc ttgtaggacc cgggattttc caaaggtgtt tgaacgtggt ccagtactac 240

atgaaggaga aagaagaaga ctctggttgg ttactggctg ggtggatcaa ggaaacactt 300

gggagagctt tacatgagca accaatgatt tctggtcgtc ttcggaaagg ggaacgaaac 360

gatggagaat tggagattgt ttccaatgac tgcggcatta gactcattga ggcaaggatt 420

cagatgaatc tgtcggattt tcttgatttg aaacaaaggg aagatgctga agctcagctt 480

gttttctgga aagatattga tgagcaaaac ccacagttct ccccactctt ttatgttcag 540

gttactaatt tccagtgtgg tggatattca attgggatta nctgcagtat tcttctggca 600

gatcttttgt taatgaaaga attcct 626

<210> 49
<211> 644
<212> DNA
<213> Gossypium arboreum

<220>
<221> misc_feature
<222> (585)..(585)
<223> n = unknown

<400> 49
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acttgaatgt tgtgcttgtt cttgctctag tagtggttca agctactgca aggaatgtgc 120

ctagcgatgc tgctggtctc aatgaccaaa agaacctcct cacatacggt ggcattggcg 180

gctactctgg catgggttca aatggcatgc caatgggtgg agttgggagt gttggtggta 240

tgactggcct tggtggtaca ggtgggatgg gcgccatggt aggtgttggg tatggaggtg 300

ggcctggcgc tggtggtgga aatgaaggtg gtgttggcat tggcaatgcg cctggtgtcg 360

tccactttcc ttgaactttg ctggatggtt aaaattttaa agcaactagt ttcttgaact 420

ttgctggagg ggtttaaatt ttaaagcaac tagtctaact tacgttaaag agtaatatta 480

aagttgctct agagtgtgaa atgttttggg ttatgtgata ggtccatctt tatttttttt 540

atgtcgagtt ttcttttgtt ttgtaatcct tcattgtcgt ggttntgtag ccgacttaaa 600

gtaaataaat tgattttgac aagttaaaaa aaaaaaaaaa acaa 644

<210> 50
<211> 677
<212> DNA
<213> Gossypium arboreum

<400> 50
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atggtgtatt ttggtgcgtg tctgttgaga atccgagttg ttgtcccgtg gtattagctt 120
ctctgtcttg ctggttgcga ttgggcagtt gtgaggtcta taatcaagtg attcaaggaa 180

accgttagct tcattttact tggagaagac aaagaagcta ttgttgtgct ggacttgttc 240

ttgctttttc tctttgtatg gtgtggttta tggtttgtat tatgagtttt atatgaatag 300

aactttgaat ttggtgagaa aattaagaat gagcttggga ggagcagaag tgttgatggc 360

aatagcaggg ttgtgggcag tggttttgag gccattgatg ataaggtatg ccgtagagat 420

gagtcaaatg attggaattt ccgttaggag agttttcagt aatcctcttt ccccttccgt 480

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attttttgct agccaagaag ttagatgagt ggtagacttt ggtttaagct tatcataatt 600

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caataaaaaa aaaaaaa 677

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<212> DNA
<213> Gossypium arboreum

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acatttggca gatgaaatta tttacgaaga acaatgggat attttctgta tttgtttatc 180

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ttaatgccag aacagtttgc agatattaga agcacttgaa aaataaagct gatacagata 360

attccatatg taattctact ataatctctt tctctttgtt tctgtaatca aattccagta 420

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tcaaaatcat tataacagct gctaagtccc ac 692

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cctgggaa 788

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<213> Gossypium arboreum

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cctccccgga ccgccaaaac gtgtgcaagt gcatcaaatc ggcggtgaac ggaattccgt 300

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atctttaatt ttttaaaaaa aaaaaaaaaa aaaa 634

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caagtttcta atattctacg attcttctca 690

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<213> Gossypium arboreum

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Class I

A B

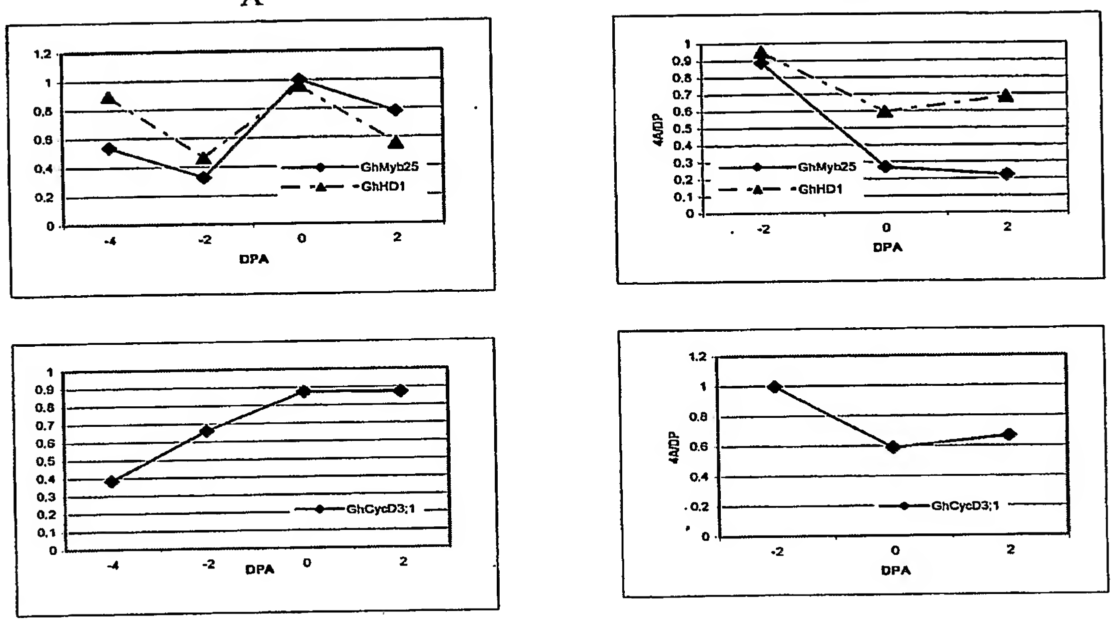


Figure 1A

Class II

A B

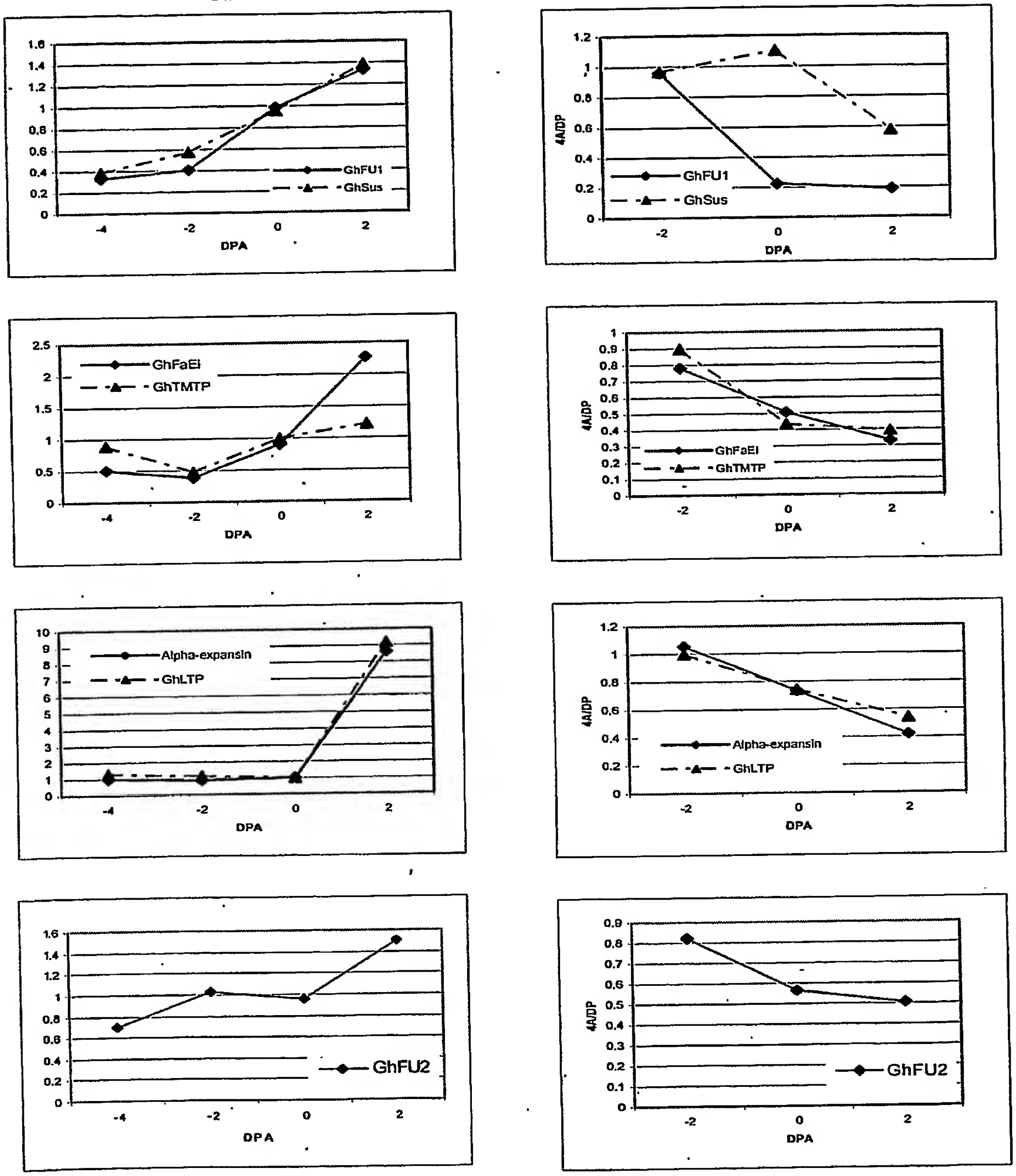
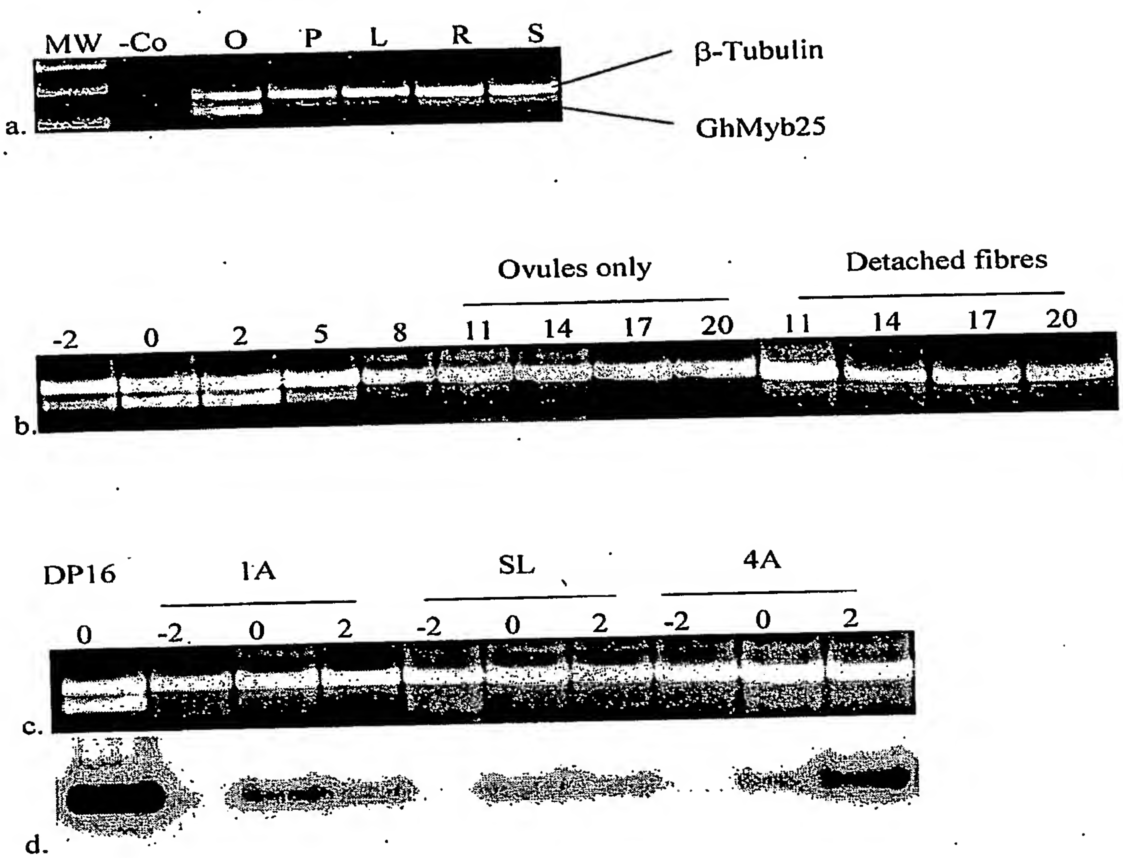


Figure 1B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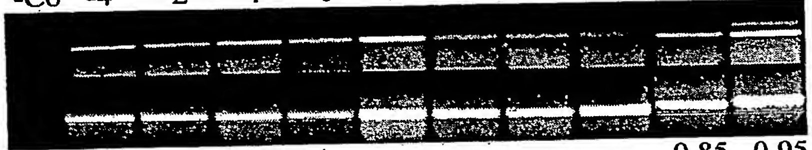
O
P
L
R
S
β-Tubulin
GhMyb25
a.
Ovules only
Detached fibres
-2
0
2
5
8
11
14
17
20
11
14
17
20
b.
DP16
1A
SL
4A
0
-2
0
2
-2
0
2
-2
0
2
c.
d.

Figure 2

-Co -4 -2 -1 0 2 4 8O 8F 0-5B 0-4A

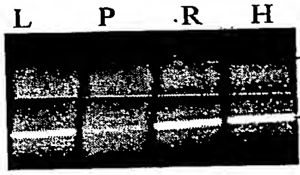


GhHD1

β-Tubulin

normalized: 1 0.85 0.95

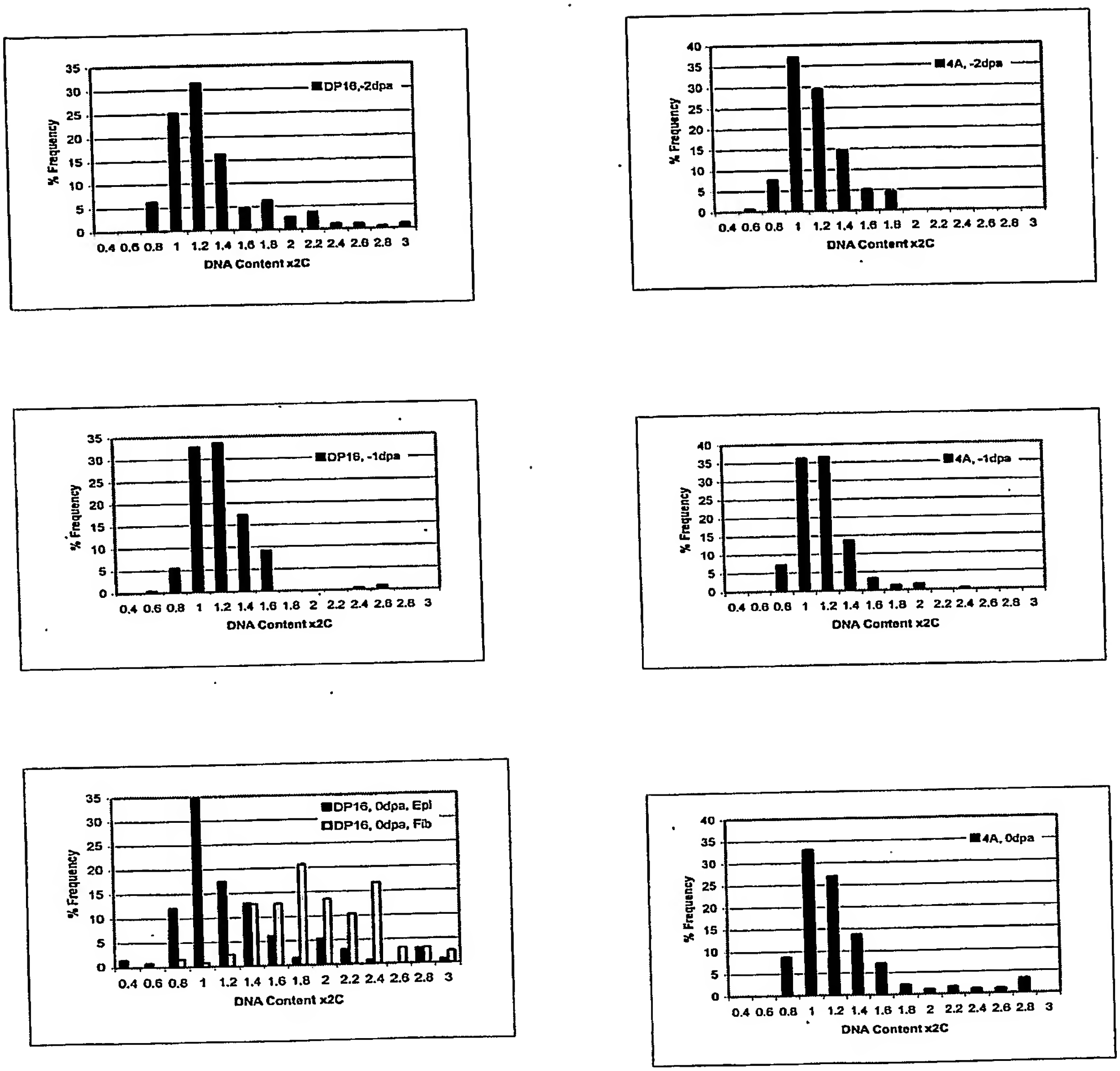
L P R H



GhHD1

β-Tubulin

Figure 3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4A, -1dpa
DP16, 0dpa, Epi
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4A, 0dpa
0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2 2.2 2.4 2.6 2.8 3

Figure 4